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TOXOPLASMIC ENCEPHALOMYELITIS *

III. A NEW CASE OF GRANULOMATOUS ENCEPHALOMYELITIS DUE TO A PROTOZOON

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In 1937 Wolf and Cowen [67] reported a case of parasitic encephalomyelitis in an infant. The causative agent was found to be a protozoon which on morphological grounds was identified as an Encephalitozoon. At the time, however, the possibility that the parasite might be a Toxoplasma was considered. In this paper reports of similar infections in infants by Jankû [21] and Torres [59–61] were cited as instances of the same disease. Later, a case reported by Richter [49] was suspected, because of its pathological picture, of being the same disease, and reexamination of his histological sections revealed the presence of parasites similar to those observed in the first 3 cases cited.[68] In reviewing Richter's case the identity of the causative parasite was reconsidered and it was pointed out that the information available at that time was somewhat in favor of the microorganism being Toxoplasma, rather than Encephalitozoon. This decision again was reached on morphological grounds, but it was indicated that the final evidence as to the identity of the microorganism would be procured only if it could be recovered from a future case and studied experimentally.

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A recent case, which came to autopsy at the Babies Hospital in New York City in June 1938, presented an opportunity for the transmission of the infection to experimental animals. A preliminary report [69] of the transmission experiments establishing the identity of the causative agent as a Toxoplasma has been made.

Toxoplasma is generally regarded as a protozoan parasite, the exact classification of which is as yet undetermined. The microorganism has been known since 1908 when it was reported independently by Splendore [55, 56] (Brazil) in the rabbit, and by Nicolle and Manceaux [41, 42] in the North African rodent, the gondi. In smears the parasites appear as well outlined, crescentic or curved masses of protoplasm 4–6μ in length and 2–3μ in width. The extremities are usually pointed, although one end may be rounded. In histological sections of fixed tissue, or under pressure, ovoid, rounded or fusiform types are more commonly seen. Each parasite contains a rounded chromatin mass situated centrally or nearer the blunter extremity. The chromatin body may appear granular or ring-like, possibly due to incomplete staining, or rod shaped or dumbbell shaped preparatory to cell division. Flagella are absent and motility has not been observed. Reproduction is by simple binary longitudinal division. In addition to the single parasites, large rounded masses of what appear to be aggregations of closely approximated parasites are also seen. Such masses are sometimes referred to as cysts although it is uncertain whether or not they possess a true cyst wall. In the cysts it is not always possible to distinguish clearly between the individual parasites which may appear to be differentiating out of a multinucleated mass of protoplasm. Such forms have been interpreted by some as indicative of reproduction by schizogenesis. It has not been possible to cultivate the parasites in artificial mediums although success has attended the use of those containing living cells.[28, 51]

In the past 30 years Toxoplasma has been described as a spontaneous infective agent in a variety of species of mammals and birds from many parts of the world. The strikingly wide geographic distribution of, and host susceptibility to these parasites is indicated by the following reports of naturally occurring toxoplasmosis: dog (Italy,[35] Brazil,[7] Germany,[72] France,[3, 39] Persia [30]);

rabbit (Brazil,[56] Senegal,[4] France,[28] Dutch East Indies,[5] Congo [52]); guinea pig (Brazil,[9] France,[37] United States [32, 51]); mouse (Italy,[53] France [38]); rat (Italy [54]); gondi, *Ctenodactylus gondi* (Tunisia [42, 43]); squirrel (England [13]); mole (Japan [48]); snake (London Zoological Garden [47]); lizard (France [14]); the fossa, *Cryptoprocta ferox* (London Zoological Garden [47]); wombat (Wellcome Bureau, London [15]); monkey, *Stentor senilicus* (Guiana [57]); baboon, *Cynocephalus* (France [29]); chimpanzee (France [22]); domestic pigeon (Brazil,[7] India [34*]); English sparrow, *Passer domesticus;* starling, *Sturnus vulgaris;* canary, *Serinus canarius* (United States [18, 19, 31, 70]); siskin (Germany [64]); and various other birds from the same and other lands.†

The morphology of the microorganisms and the histopathology of the disease produced in the animals are often very inadequately described. Some of the animals may not have had spontaneous toxoplasmosis since they were inoculated for other purposes and may have inadvertently received injections of tissues which were Toxoplasma-infected. Such biological characteristics as might be studied by transmission of the infection are lacking in many instances. Most of the reports however, are sufficiently docu-

* In these reports the parasites were not designated as Toxoplasmata by the author, but subsequent writers [33, 66] have generally so regarded them.

† These include the following: Brazil [58]: white-throated seed-eater (*Sporophila albogularis*), Andean white throat (*Brachyspiza capensis*), white-bellied swallow (*Atticora cyanoleucus*), yellow finch (*Sicalis flaveola*), palm tanager (*Tanagra palmarum*), Dominican cardinal (*Paroaria larvata*), red rump tanager (*Rhamphocelus brasilius*). Brazil [8]: rufous-bellied thrush (*Turdus rufiventris*), blue-black grassquit (*Volatinia jacarini*), (*Aaptus etiopi*), tyrant flycatcher (*Pitangus sulphuratus*), white-crested elaenia (*Elaenia albiceps*), king vulture (*Gypagus papa*). Brazil [49]: tanager (*Tanagra sayaca*). Gambia [59*]: African vulture (*Neophron monachus*). India [34*]: sparrow. France [23*, 25, 33]: Java sparrow (*Padda oryzivora*), waxbills (*Estrilda phoenicotis*), (*Lagonosticta senegala*), weaver bird (*Quelea erythrops*), fire finch (*Pyromelona francisca*), chaffinch (*Fringella coelebs*), yellow babbler (*Liothrix luteus*). London Zoological Garden [47]: fruit pigeon (*Carpophaga concinna*), pied bush chat (*Pratincola caprata*). United States [45*]: sparrow. United States [30]: catbird (*Dumatella carolinensis*), chipping sparrow (*Spizella passerina*), kingbird (*Tyrannus tyrannus*), red-eyed towhee (*Pipilo erythrophthalmus*), song sparrow (*Melospiza melodia*), swamp sparrow (*Melospiza georgiana*), Baltimore oriole (*Icterus galbula*), cowbird (*Molothrusater*), Savannah sparrow (*Passerculus savanna*). United States [71]: house finch (*Carpodacus mexicanus frontalis*). Argentina [50]: canary (*Serinus canarius*). Japan [62, 63]: white-eye (*Zosterops palpebrosa peguensis*), paddybirds (*Munia malaca, Munia maja, Munia atricapilla, Munia topela, Ploceus baya, Aidemosyne malabarica*), bamboo finch (*Erythrura prasina*), Java sparrow (*Oryzornis oryzivora*). Italy [17]: European tree sparrow (*Passer montanus*), Italian house sparrow (*Passer italiae*). Germany [44]: English sparrow (*Passer domesticus*), green finch (*Ligurinus chloris*), linnet (*Cannabina linota*).

mented and support the impression of the wide distribution of the infection.

Following the usage of Nicolle and Manceaux, who named their parasite *Toxoplasma gondii*, many subsequent observers have referred to each newly reported strain as *Toxoplasma cuniculi*, *T. musculi*, *T. columbae*, and so on, according to the host in which it was discovered. As a number of authors [12, 66] have pointed out, however, there is no morphological or other means of distinguishing between the various named forms of the parasite, and it may well be that they represent a single species capable of infecting many hosts. This low host specificity has been demonstrated experimentally on many occasions by inoculation of Toxoplasma-infected tissue into heterologous animal species. Thus, *T. gondii* [12, 24, 43] has been found pathogenic for the mouse, guinea pig, pigeon, rabbit, mole, shrew, dog, Java sparrow and cat; *T. cuniculi* [28] for the guinea pig, mouse, pigeon, chick, sparrow and other small birds; *T. canis* [39, 40] for the rabbit, guinea pig, mouse, sparrow and pigeon; *T. caviae* [9, 51] for the pigeon, mouse, rabbit, chick and chicken. Nothing is as yet known of the natural mode of transmission of the infection in animals or of the existence of intermediate vectors. In naturally infected animals the parasites have commonly been found free or in leukocytes in inflammatory parenchymal lesions and exudates in a variety of tissues, notably the brain, spinal cord, spleen, lung and liver. They have also been observed occasionally in the blood, bone marrow, intestinal mucosa, lymph nodes, heart, kidney, pancreas, omentum, mesentery and pleural exudate. The pathological changes in spontaneously infected animals have in most instances not been thoroughly described. The lesions most commonly encountered are small focal inflammatory nodules, with or without necrosis, in the spleen, lung and liver. The infiltrating cells are predominantly lymphocytes and large mononuclears. Comparable changes have been noted in the brain although there is little or no reaction to the presence of the parasites in this organ in some instances. The intestine may show ulceration. The kidney generally shows no pathological changes. The lesions in experimental toxoplasmosis, as described in the literature, and as we were able to observe them in rabbits and mice inoculated with a strain of Toxoplasma of animal origin [51] from the Rockefeller Institute of New York,

kindly furnished us by Drs. Sabin and Olitsky, may be briefly summarized. Intracerebral inoculation of rabbits results in chronic inflammatory foci in the leptomeninges, parenchyma, ventricular walls and choroid plexuses. The infection may spread down to the spinal cord. The lesions contain lymphocytes and mononuclear cells with fibrin and polymorphonuclear leukcocytes when necrosis occurs. Plasma cells are common in the chronic lesions. Productive changes, such as multiplication of leptomeningeal cells, endothelial proliferation with production of new capillaries, formation of small granulomas of capillary origin, and proliferation of microglia and fibroblasts, are of frequent occurrence. The parasites occur free, in cysts, and intracellularly, in leptomeningeal cells, large mononuclear cells, endothelial cells of capillaries, epithelioid cells of the granulomas, phagocytes, ependymal and choroid epithelial elements, and rarely in nerve cells within the focal lesions. They are most numerous in the necrotic foci, although they may occur in considerable numbers about involved blood vessels, spreading into the parenchyma without much initial reaction. There are focal inflammatory and, at times, necrotizing and productive lesions in other organs. The lungs, liver and spleen, and less commonly the heart, adrenals, kidneys, intestines and lymph nodes are affected. Parasites are present in these lesions. Combined intracerebral and intraperitoneal inoculation of mice results in essentially the same picture with an associated peritonitis. Parasites may be found free and in large mononuclear cells in the peritoneal exudate. Infection also develops after inoculation by other routes with widespread dissemination and variations in the intensity and localization of the lesions in various organs.

The following is a description of the clinical and pathological changes observed in an infant from whom Toxoplasma was recovered. In view of the rarity of this condition a complete report of the case is presented.

Report of Case

Clinical History: C.D. (Babies Hospital, New York, No. 552107), a white male infant, was delivered at term by Cesarean section on May 23, 1938.

Both parents were in good health and had always resided in or near New York City. No history of contact of the parents with rabbits or other animals could be elicited. The mother had never eaten rabbit meat. The apartments

in which she had resided for several years before admission to the hospital were free of mice and rats. No pets, including birds, were kept in the home. The father was 32 years of age. The mother, a primipara 31 years of age, had been under continuous observation in the hospital for the last 3 months of her pregnancy as a suspected case of placenta praevia. During this period her temperature, pulse, respiration and blood pressure remained within normal limits. Her blood Wassermann test was negative, and the course of pregnancy was uneventful except for slight vaginal bleeding during the last months. Several days before the expected date of delivery there was spontaneous rupture of the membranes with a profuse discharge of yellowish fluid. During pelvic examination a large amount of thin meconium escaped. In view of the placenta praevia, it was decided to deliver the child by Cesarean section. This was performed under nitrous oxide-ether anesthesia. Recovery from the operation was uncomplicated. The placenta and membranes were unfortunately not kept for examination.

At birth the infant was moderately asphyxiated but respiration was described as spontaneous. Crying was not vigorous. The infant weighed 3050 gm. and measured 50 cm. in length. The head measured 33.5 cm. in circumference, the chest 31 cm., and the abdomen 33 cm. On the 3rd day of life the infant had a right-sided convulsive seizure, lasting 3–4 minutes, with jerking of the hands, twisting of the mouth, and rolling of the eyes to the right. During the attack the respirations were shallow and rapid. There was no rigidity of the neck or bulging of the anterior fontanelle. The body temperature remained normal. Following the convulsion the infant was apathetic and respirations were irregular, shallow and rapid. A left-sided Horner's syndrome was observed. No gross hemorrhages were noted in the fundi. There was a normal withdrawal reaction to pin prick. The tendon reflexes were absent in the legs and hypoactive in the arms.

During the next few days of life the child's condition improved somewhat. There were repeated convulsive seizures but these gradually became less frequent. Feedings were taken fairly well, although in general the infant was drowsy and lethargic. By the 2nd week convulsions ceased, although weakness and a left enophthalmos persisted. A roentgenogram of the lumbosacral spine at this time showed no abnormalities. Lumbar puncture yielded bloody fluid but the relatively uncontaminated portions of the fluid appeared xanthochromic.

Physical examination on the 17th day of life showed retraction and turning of the head toward the right. The eyes deviated to the right and at times there was nystagmus toward the right. The upper extremities were spastic and the hands clenched, but the tendon reflexes were not increased. No thoracic breathing could be made out. The abdominal reflexes were absent, but sluggish cremasteric responses could be obtained. The lower extremities were withdrawn on painful stimulation. Babinski reflexes were not elicited. Response to pin prick was definitely less marked below the neck than on the face and scalp. The spleen was just palpable. The bladder was distended and there was dribbling of urine. The fontanelles were not tense nor was there any separation of the cranial sutures. It was felt that the clinical signs pointed to both spinal cord and cerebral injuries with the more severe injury in the spinal cord. The patient was discharged from the hospital at the age of 18 days.

At home there was at first no change in the infant's condition. On the 22nd day of life, however, the mother noticed a seizure in which the child's arms became stiff and remained straight and stiff by his sides. Following this episode he seemed weaker. On the 26th day vomiting began and although the mother diluted the formula and fed it in small amounts, the feedings were not retained. For this reason the infant was readmitted to the hospital after having been at home for 10 days.

As contrasted with examination several days previously, the infant's condition was poor. Respiration was quite irregular and labored, and appeared to be almost entirely diaphragmatic in type. There was moderate cyanosis and a grayish tint to the skin. No spontaneous movements below the neck were observed. The arms were pronated and held in extension at the elbows, and the fingers were kept flexed. Attempts to elicit the deep reflexes in the lower extremities resulted in flexion of the thighs and legs, and dorsiflexion of the feet. The abdominal reflexes were absent. There appeared to be insensitiveness to pain below the neck. Both liver and spleen were palpable but thought by the examiner to be of normal size.

Laboratory Data: Examination of the urine on June 20, 1938 showed a trace of albumin, no sugar, and microscopic examination showed 6–30 polymorphonuclears per high power field with occasional erythrocytes. A blood count on June 20th showed erythrocytes 5,970,000 per cmm., total leukocyte count 8000 per cmm.; polymorphonuclear leukocytes 61 per cent, lymphocytes 33 per cent, eosinophils 5 per cent, and monocytes 1 per cent. Examination of the spinal fluid on June 21st showed a pressure of 140 mm. of water. Respiratory oscillations were present but there was no response to compression of the jugular vein on either side. Three cc. of slightly cloudy, distinctly xanthochromic fluid were removed. This clotted promptly and gave a 4 plus Pandy reaction. The benzidine test was negative. Microscopic study of the fluid was not made. A blood Kahn test on June 23rd was negative.

Terminal Clinical Observations: In view of the suspected involvement of the cervical cord with subarachnoid 'block,' a cisternal puncture was attempted. About 3 drops of rather viscid, clear, slightly xanthochromic cerebrospinal fluid were obtained which gave a positive benzidine test and a 4 plus Pandy reaction. About 0.1 cc. of lipiodol was injected. Fluoroscopy and roentgenographic examination of the spine at intervals following this procedure showed all of the lipiodol to remain above the level of the second cervical vertebra, suggesting a 'block' at this point. During the last few days of life the infant's temperature fluctuated between 96° and 101.2° F. The cry was weak and respirations were of the Cheyne-Stokes type.

Examination of the fundi revealed an irregular reddish brown area in each macular region about 1 disc-diameter in size on the right, and somewhat smaller on the left. These changes were interpreted as resulting from hemorrhage.

On June 23, 1938, the infant became markedly cyanotic with rapid shallow respiration. At death the baby was 31 days of age. The clinical diagnosis was multiple injuries of the central nervous system with compression of the cervical spinal cord by hematoma.

Autopsy Report

Postmortem examination was performed 4½ hours after death and was limited to examination of the brain, spinal cord and posterior hemisphere of the right eye.

The surfaces of the cerebral hemispheres presented numerous focal lesions (Fig. 1) varying from a few mm. to approximately 2 cm. in diameter. In these areas the cortex was depressed, pitted and discolored yellow. This tissue was in most instances considerably softer than that of the surrounding unaffected gyri, and the overlying leptomeninges were thickened and grayish yellow in color. The most prominent lesions involved portions of the superior and middle frontal gyri, the frontal and temporal poles, the precentral and postcentral gyri, the superior parietal lobule and the lateral occipital gyri bilaterally. Most of the gyri on the medial and ventral surfaces showed similar focal lesions. The cerebellum and brain stem appeared normal externally. The leptomeninges about the basal cisterns were slightly grayish and opaque.

Coronal sections of the left cerebral hemisphere showed that the superficial lesions consisted of well demarcated zones of cortical softening (Fig. 2). The center of each was slightly sunken, finely cystic, and yellowish or grayish yellow in color, while its margin was sharply outlined, grayish and more prominent. The larger lesions extended from the cortex into the subcortical white matter, and at times into the centrum ovale. On incising some of the softened areas the knife passed through gritty material. A large cyst, 5 cm. in length anteroposteriorly, 1.5 cm. in diameter in its greatest cross section, and lined by necrotic discolored parenchyma, was present in the white matter inferomedial to the atrium and occipital horn of the left lateral ventricle.

Single and conglomerate foci of softening similar to those in the cortex involved portions of the island of Reil, and lenticular and caudate nuclei. The thalamus and hypothalamus appeared grossly unaffected.

The changes in the right cerebral hemisphere were much the same as those in the left, except for the absence of large cysts.

The lateral and third ventricles were slightly dilated. Their walls were smooth and glistening, except in the inferomedial

angles of the lateral ventricles where there were areas of yellowish discoloration denuded of ependyma.

Section of the cerebellum revealed no gross abnormalities, while the brain stem contained numerous lesions. There were numbers of soft, irregular, yellowish white areas in the midbrain, each bordered by a grayish translucent marginal band. The largest area was located in the right cerebral peduncle and measured 5 by 2 mm. in cross section. The others were present in the oculomotor nuclei and ventral portions of the red nuclei. The aqueduct of Sylvius was of normal size. In the cephalic portion of the pons, at the junction of the tegmentum and reticular area, was an ovoid, translucent grayish area 5 by 3 mm. in cross section, traversed by fine yellow, curving and interlacing lines (Fig. 7). Small, cream colored, sharply marginated lesions, pinpoint in size, were scattered through the reticular zone and in the regions of both fifth nerve nuclei. In the cephalic portion of the medulla two dull white areas with gray centers and scalloped edges were found in the restiform bodies and fifth nerve nuclei bilaterally, the right measuring 4 mm. and the left 2 mm. in diameter. At their margins white pin-point nodules were seen. The rest of this portion of the medulla was glassy in appearance and showed slight obscuration of its markings. In the midmedulla there were numerous lesions similar to those described in the midbrain. Many of those on the right side were softened centrally. The largest lesion was 10 by 5 mm. in cross section, oval, sharply marginated, grayish white in color, and involved almost the entire left side of the medulla at this level. At the junction of the middle and lower thirds of the medulla a syrinx measuring 4 mm. in length and 1 mm. in transverse diameter was present. It was lined by a narrow band of yellowish and gray translucent tissue, widest at the lateral border of the cavity. The right half of the lower medulla was soft to palpation and its architectural markings were obscured by gray and cream colored mottling.

The cervical segments of the spinal cord were markedly swollen, the greatest swelling occurring in the region of the cervical enlargement, which was firm and almost twice its normal size. The leptomeninges over its cephalic portion were slightly clouded. The first three segments of the thoracic cord were shrunken to about one-half of their normal diameter, and were softer than the ad-

jacent parenchyma. The leptomeninges over them were opaque and yellowish. There was a similar change in the seventh, eighth and ninth thoracic segments and overlying pia-arachnoid. The lowest thoracic segment was slightly swollen. The lumbosacral segments of the spinal cord, the nerve roots and the cauda equina appeared normal. Except in the regions where they were specifically noted as abnormal, the leptomeninges were thin and translucent. On section of the swollen cervical cord the parenchyma was observed to be translucent and gray, and the line of demarcation between gray and white matter was obliterated (Fig. 3). Section of the shrunken upper segments of the thoracic cord (Fig. 4) revealed that their normal markings were obscured. The tissue was gray and translucent and in it there were a number of punctate yellow and brownish nodules. In the seventh to ninth thoracic segments (Fig. 5) an area of grayish and light brown glistening tissue measuring 4 by 2 mm. in cross section was present in the central portion of the parenchyma obscuring all the gray horns. At the margins of this zone the tissue was studded by yellow punctate nodules 1–2 mm. in diameter. In the upper lumbar segments (Fig. 6) the outlines of the ventral horns were well preserved. The posterior horns, however, were obliterated by grayish tissue which passed into the posterior columns and parts of the lateral columns as well. Yellowish nodules, 1 mm. or less in diameter, were occasionally seen in the white matter.

Histological Examination

Leptomeninges of Cerebrum: Over the cortical lesions the leptomeninges were markedly edematous and congested. There was moderate or intense infiltration by lymphocytes, plasma cells, mononuclear leukocytes, lipoid laden phagocytes and eosinophils (Fig. 11). In some areas the last were almost as numerous as the plasma cells. Where the parenchymal changes were most severe the leptomeningeal exudate was directly continuous with that in the cortex, obliterating the line of demarcation between pia-arachnoid and brain. There was a multiplication of leptomeningeal cells. None of the leptomeningeal vessels was occluded, but there was endothelial hyperplasia in all the capillaries, many of the arterioles and venules, and some of the small arteries and veins.

Over the unaffected gyri the leptomeninges were somewhat edematous and occasionally infiltrated by small numbers of mononuclear leukocytes and lymphocytes.

Cerebral Cortex: There was a complete loss of cortical architecture and a total destruction of all neural and glial elements in the large zones of necrosis (Fig. 8). These were occupied by a mass of eosinophilic and nuclear débris in which only a few vessels were still partially preserved. Their walls were permeated and surrounded by fibrin. Toward the margins of these areas of total degeneration, polymorphonuclear leukocytes, lymphocytes, plasma cells, lipoid laden phagocytes and eosinophils were present in moderate numbers. Frequently they were concentrated in the walls and perivascular spaces of blood vessels The parenchyma was intensely edematous and showed a considerable loss of neural and glial elements, moderate hyperplasia of capillaries, and considerable astrocytosis. The majority of the astrocytes showed intense clasmatodendrosis. Beyond the marginal zone of reaction the parenchyma was edematous and studded by numerous miliary granulomas (to be described in detail below). Between the granulomas in the gray matter there was a moderate diffuse loss of ganglion cells, and many of the remaining cells were either swollen and vacuolated or pyknotic. The oligodendroglia were swollen and the astrocytes hypertrophied and fibrillary.

In some of the severely necrotic areas cyst formation occurred. Within the cavities lipoid laden phagocytes lay in a meshwork of hyperplastic capillaries, the walls of which were infiltrated by small numbers of plasma cells, lymphocytes and mononuclear leukocytes. Fibroblasts derived from the capillary walls were present. There was multiplication of microglia cells at the margins of the cavities and of the necrotic zones. They gave rise to numerous rod cells and transition forms of lipoid laden phagocytes. In relation to the cysts, astrocytosis was usually more prominent, the cells consisting of plump astrocytes, some of which were binucleated or multinucleated.

A striking feature of the process was the marked calcification of necrotic material in some areas (Fig. 9). This involved either the majority of the cortical layers, resulting in a broad band of calcification, or consisted of scattered calcific material, such as

was seen at the margins of the cysts. The bands of calcification often stopped abruptly. The calcium occurring in coarse granules was deposited in clusters or diffusely. Considerable numbers of calcified cells (Fig. 15), the majority of which appeared to be nerve cells, were identified. The margins of these were sharp and the nuclei remained unencrusted. Short undulating bands of calcium of varying thickness had the appearance of encrusted dendrites or axones.

The cortex adjacent to these foci of severe cortical necrosis, inflammation and calcification was often surprisingly well preserved. Single granulomas were occasionally encountered in these areas, most frequently in the zonal or pyramidal layers (Fig. 10). The cortical architecture was normal but there was a diffuse edema and a slight diffuse loss of ganglion cells. The remaining neural elements had undergone complete chromatolysis, and some were swollen, vacuolated or pyknotic. No myelin sheaths were present in the gray matter, but moderate numbers of unchanged axones were observed. There was a mild generalized astrocytosis with thickening of the external glial membrane near the focal lesions.

Cerebral White Matter: Where the cortical lesions were most severe, inflammation and necrosis extended into the subcortical white matter and in places into the centrum ovale. Here amorphous débris was found in place of normal structures, and at its margin was a band of intensely edematous, partially degenerated white matter, moderately infiltrated by cells similar to those seen in the cortex. The capillaries in this zone were hyperplastic and there was a considerable astrocytosis, many of the astrocytes being partially degenerated. Numerous miliary granulomas were present at the margins of the zones of infiltration and in the relatively intact white matter subjacent to purely cortical lesions. The oligodendroglia were markedly swollen. Small numbers of myelin sheaths were seen in the subcortical white matter and centrum ovale. They were widely separated, but they, as well as the axones, appeared normal. The subependymal tissue about the lateral ventricles showed a marked astrocytosis. This broadened subependymal glial mat was mildly infiltrated by plasma cells and lymphocytes. The infiltration varied in intensity and where it was greatest granulomas were present. Small deposits of calcium

were occasionally seen. The walls of the lateral ventricles were denuded of ependyma at some points.

Corpus Striatum: There were large areas of necrosis involving the globus pallidus, putamen and caudate nucleus. Centrally these were filled with eosinophilic débris and degenerating lymphocytes, mononuclear leukocytes and polymorphonuclear leukocytes. Focal collections of such cells clustered about persisting capillaries. The degeneration passed into the adjacent internal capsule and centrum ovale. Where it reached the surface of the caudate nucleus the wall of the lateral ventricle was completely denuded of ependyma except in the lateral angle (Fig. 13). In the superficial periventricular necrotic material there were small numbers of degenerating mononuclear leukocytes and occasional clusters of calcium granules. At the margins of the totally degenerated areas the inflammatory, glial and vascular changes were similar to those described in the cortex. Granulomas in moderate numbers were scattered through the rest of this region. The nerve cells in the preserved areas showed complete chromatolysis and occasional vacuolization. Most of the myelin sheaths, not directly in the zones of necrosis, were well preserved.

Thalamus and Hypothalamus: The nerve cells in the thalamus were well preserved and contained a normal amount of Nissl substance. Numerous granulomas were scattered through the parenchyma, and some were present in the thickened subependymal glial mat in the wall of the third ventricle. The ependyma lining the latter was well preserved, although there were numerous ependymal granulations.

Cerebellum: The leptomeninges over the folia were slightly congested and contained occasional lymphocytes and monocytes. There were no abnormal changes observed within the folia proper. Occasional granulomas were found in the central white matter of the cerebellum and subependymal tissue of the wall of the fourth ventricle.

Midbrain: The leptomeninges were congested and contained small numbers of mononuclear leukocytes. The aqueduct of Sylvius was slightly reduced in size due to the presence of many flat ependymal granulations. Numerous granulomas similar to those in the cerebrum were scattered in the substantia nigra, cerebral peduncles, colliculi and periaqueductal region. A group

of large nerve cells in one colliculus was partially calcified. The rest of the nerve cells in the nuclei at this level showed no abnormal changes, and the axones were well preserved. The myelin sheaths appeared normal. There was a slight astrocytosis about the granulomas.

Pons: There were many more granulomas in this portion of the brain stem than in the midbrain, and they occurred in both the reticular and the tegmental regions, as well as in the middle cerebellar peduncles. A large area of diffuse infiltration was encountered in the midline of the dorsal half of the reticular zone. Here the tissue was quite edematous, showed mild astrocytosis, and was moderately infiltrated by lymphocytes, plasma cells, mononuclear leukocytes and occasional eosinophils. Some of the vessels showed mild endothelial hyperplasia and infiltration of their walls. The nerve cells were fairly well preserved. In foci within the zone of inflammation the process was more intense. Here there was a concentration of infiltrating cells with satellitosis and varying degrees of degeneration in some of the ganglion cells. In these foci capillary hyperplasia and microglial proliferation were also seen. Granulomas were concentrated near the margins of the infiltrated zone and involved the nearby pyramidal tracts. There were small numbers of ependymal granulations in the floor of the fourth ventricle and the subependymal glial mat was thickened and contained granulomas. The leptomeningeal changes were similar to those about the midbrain.

Medulla: In the cephalic portion of the medulla there were disseminated miliary granulomas. In the ventral half of the midmedulla diffuse infiltration and partial degeneration were present. In addition to infiltrating cells similar to those described elsewhere, there were small numbers of lipoid laden phagocytes. The exudate showed focal and perivascular concentration. All of the neural and glial elements had disappeared. At the margins of the necrotic and inflammatory zone were vascular hyperplasia and a concentration of granulomas. Degeneration and inflammation were even more extensive in the caudal portion of the medulla where only a narrow band of relatively well preserved tissue containing numerous granulomas was present at the periphery. A large central cavity was present in the necrotic zone. Most of the neural elements had disappeared in the degenerated area and only

a few were preserved in the nuclei at its edges. Axones and myelin sheaths were well preserved in the marginal tissue but had disappeared elsewhere.

Choroid Plexus: Especially in the lateral ventricles near the glomus, there was a moderate amount of exudate between the villi of the choroid plexus. This was composed of mononuclear leukocytes, lymphocytes, and occasional polymorphonuclear leukocytes and strands of fibrin. The choroid tufts were hyperemic and their central connective tissue cores edematous. Eosinophils, scattered in small numbers beneath the choroid epithelium, were present in nearly every villus. There were fewer lymphocytes and polymorphonuclear leukocytes and these were usually found deeper in the core of the tuft. Occasionally there was a more cellular inflammatory focus, and over this area the choroid epithelium had disappeared in places.

Spinal Cord: Upper Cervical Region: The spinal roots showed no abnormal changes. The leptomeninges were mildly infiltrated by lymphocytes, mononuclear leukocytes, polymorphonuclear leukocytes and eosinophils. This mild infiltration passed into the dorsal medial septum and showed focal concentration over the more severe parenchymal lesions where it was associated with hyperplasia of leptomeningeal cells. The leptomeningeal vessels were patent although some of the smaller ones showed endothelial and adventitial hyperplasia. The most marked parenchymal changes occurred in the columns of Goll and were similar to those in the lower medulla, except for the absence of a syrinx. There was marginal astrocytosis and granulomas were present in the columns of Burdach. A small number of granulomas was scattered through the remainder of the white matter. There was generalized edema, most marked in the anterior horns where the nerve cells were pale staining and a few partially degenerated. Polymorphonuclear leukocytes and lymphocytes in moderate numbers were present here (Fig. 14). The central canal was distended by amorphous material in which polymorphonuclear leukocytes, lymphocytes and mononuclear leukocytes were seen. There was a total loss of myelin sheaths and axones in the posterior columns. At the margins of the degenerated zones swollen fragmented myelin sheaths were seen and phagocytosis of myelin fragments by large mononuclear cells occurred.

Cervical Enlargement: The spinal cord was considerably swollen at this level due to intense edema. The posterior columns showed even greater degeneration than in the preceding level with extension of necrosis and inflammation into the posterior horns and parts of each lateral column. Most of the nerve cells in the posterior horns and the medial portions of the anterior horns had either disappeared or showed marked degeneration. The remainder of the nerve cells had undergone complete chromatolysis. Granulomas were present, not only in the white matter at this level but also in the anterior horns. There was a loss of myelin sheaths in the posterior and lateral columns and a mild diffuse loss in the anterior columns. The other changes were similar to those at the preceding level.

Upper and Lower Thoracic Levels: These corresponded to the macroscopically shrunken regions. There was almost complete necrosis of the parenchyma which was collapsed and devoid of architectural markings. The leptomeningeal infiltration in these areas contained a greater number of plasma cells. In places the exudate passed directly into the parenchyma so that the line of demarcation between pia-arachnoid and cord proper was often lost. Granulation tissue invaded the posterior columns from the leptomeninges. Almost all of the remaining tissue consisted of cellular débris and occasional hyperplastic capillaries. All the neural and neuroglial elements had disappeared. In a few strips of partially preserved marginal white matter, mild inflammatory and productive changes, fragments of degenerated myelin, and large clusters of lipoid laden phagocytes were seen.

Lumbar Cord: In the upper lumbar segments there was widespread necrosis of the posterior columns, dorsal portions of the lateral columns, posterior horns, parts of the anterior horns, and central gray matter and commissures (Fig. 18). The changes in these areas were similar to, but less intense than, those in the severely degenerated areas of the thoracic cord. In the lower lumbar segments and in the sacral cord there was a mild leptomeningitis. Numerous miliary granulomas were scattered throughout the parenchyma, especially in the white matter (Fig. 17). The majority of the nerve cells were normal. The descending tracts showed degeneration, while the ascending tracts were well preserved.

Granulomas: Each of the granulomas referred to above was composed of large polygonal, round, stellate or irregularly shaped cells (Fig. 12). These had abundant eosinophilic, homogeneous or coarsely granular cytoplasm. Their nuclei were spherical, oval or irregular, stained deeply and contained a moderate amount of coarsely granular chromatin. In some granulomas few, if any, infiltrating cells were seen. In others, lymphocytes, mononuclear leukocytes and occasional eosinophils or plasma cells were distributed among the outer epithelioid cells. Hyperplastic capillaries were often seen at the margins of these lesions or embedded in them. Their plump endothelial cells seemed to be the precursors of the epithelioid cells of the granulomas. No giant cells or any central necrosis of the lesions were encountered. In the gray matter, well preserved nerve cells were frequently found at the margins of the granulomas. Myelin sheaths were deflected at their margins or often passed directly through the granulomas unchanged. Small numbers of axones were similarly affected. There was a moderate astrocytosis at the margins of the lesions in some areas (Fig. 16), and occasionally persisting astrocytes mingled with the outer epithelioid cells.

Right Eye: One portion of the retina (Fig. 19) showed edema of all its layers and distortion of the stratum opticum, ganglionic, and inner molecular layers. The capillaries in this zone had undergone endothelial hyperplasia. Many nerve cells in the ganglionic layer were partially degenerated. The layer of rods and cones was markedly necrotic. The pigmented layer was partially disrupted and the destruction of its cells gave rise to much extracellular pigment. The choroid was congested, showed capillary hyperplasia, and was infiltrated by plasma cells, lymphocytes and eosinophils. The leptomeningeal sheath of the optic nerve showed a similar mild infiltration.

Microorganisms: Parasites (Figs. 20–27) were present in the lesions of the central nervous system and eyes, and occurred in two forms. They were commonly seen as single bodies, ovoid, oval, pyriform or rounded in shape, either free in the tissue or within the cytoplasm of cells. In material fixed in 10 per cent formalin, refixed in Zenker's solution, and embedded and cut in paraffin, in sections 4–5μ in thickness, the majority of the organisms measured 2–3μ in length, and 1.5–2μ in width. More fell below this

range than rose above it. Each of the parasites was distinctly outlined and had a more deeply staining margin. A round, oval or band-like chromatin mass, the largest of which was one-third the size of the corpuscle, was present at or near one pole. Rarely the microorganisms were quite slender and lunate or scimitar-like in shape, and in these, lunate or comma shaped as well as band-like chromatin masses were more frequent. Two chromatin masses were occasionally encountered. One might be smaller and situated at the opposite pole of the cell. More often they were equal in size, symmetrically placed with their long axes parallel, and the parasites containing them probably represented dividing forms. The edges of the chromatin bodies were sharp and usually stained more deeply than their central portions. The cytoplasm at their margins was often lighter staining. No rod-like chromatin body (kinetoplast) was present, nor could a polar capsule or filament be identified. No budding was seen. The staining reactions were similar to those of the microorganism described in our 1st case.[67]

The intracellular parasites were present either singly or in considerable numbers, usually in mononuclear cells, often in epithelioid cells of the granulomas, less frequently in polymorphonuclear leukocytes and rarely in the endothelial cells of capillaries. None was found in nerve cells or eosinophils. The intracellular microorganisms lay in the cytoplasm of the parasitized cell, sometimes within a vacuole. Such cells frequently showed degeneration varying from swelling and vacuolization of the cytoplasm to extrusion of the nucleus, disruption of the cytoplasm and liberation of the microorganisms. Often the parasitized cells were surprisingly well preserved. The extracellular parasites showed varying degrees of pallor and degeneration in the necrotic areas of the parenchyma, but were well preserved elsewhere. In the areas of calcification some of the single calcific granules and a few of the clusters suggested encrusted microorganisms. The shape of the granule and an unstained area corresponding to the chromatin body were the identifying features.

The parasites were also found in compact, round or oval clusters which varied in size from 8 by 8.5μ to 16 by 19.5μ. The organisms in these masses were closely packed, often smaller than the free corpuscles, and on close inspection their individual outlines were

easily discernible in thin sections. In some clusters, however, the cytoplasm seemed to form a continuous syncytial mass, although this was not common. Such compact groups of parasites had the appearance of cysts, but they may simply have represented cells which had lost their nuclei and were completely occupied by microorganisms. Rarely a flattened nucleus was present at the margin of the cluster. At the margins of the cysts a matrix less dense than the cytoplasm of the parasites was present in the spaces between them. An outer membrane appeared to envelop the cluster. This was sharply outlined, refractile, but not doubly contoured, and as was noted in the description of our 1st case, may represent the denser appearing edge of the matrix. The majority of the cysts lay free in the tissue. Cysts were more often encountered in comparatively normal marginal tissue at the edges of the parenchymal lesions, particularly in the retina.

The cysts and individual organisms were never present in normal tissue distant from the lesions. They were found in small numbers in the leptomeningeal exudate, and in great numbers in the parenchymal lesions of the brain and spinal cord. They were occasionally seen in the ventricular exudate and in that of the spinal canal. Only a few were encountered in the choroid plexus, walls of the blood vessels and between ependymal cells. They were seen once within the lumen of a spinal leptomeningeal arteriole.

No other organisms were detected in any of the sections stained by a variety of methods.

Summary of Case

A white male infant, delivered at full term by Cesarean section, became ill at 3 days of age and developed convulsive seizures, disturbances in respiration, and symptoms of involvement of the spinal cord with subarachnoid 'block' in the cervical region. Terminally, irregular reddish brown areas were observed ophthalmoscopically in each macular region. The infant died at the age of 31 days. Autopsy revealed a widespread encephalomyelitis characterized by multiple focal areas of inflammation and necrosis, and disseminated miliary granulomas. There was localized leptomeningitis in relation to the superficial parenchymal areas of inflammation. Cystic degeneration occurred in some of the lesions while others, especially those in the cerebral cortex, showed a dis-

tinct tendency to become calcified. The inflammation and degeneration in the spinal cord resulted in a marked swelling of the lower cervical segments, sufficient to account for the subarachnoid 'block' observed during life. The right eye contained a localized zone of chorioretinitis. A protozoan parasite was present, often in great numbers, in the leptomeningeal and parenchymal exudates, in the granulomas and in the lesions of the choroid and retina.

Discussion

The case reported above is the 5th recorded instance of a new disease entity, a form of granulomatous encephalomyelitis of infants. The clinical history resembles that of our 1st case in the occurrence of symptoms soon after birth, the manifestations of diffuse involvement of the central nervous system, the evidence of focal chorioretinitis, the short course and the fatal outcome. That the 2 cases are instances of a single disease is further indicated by the similar pathological changes: a widespread encephalomyelitis characterized by inflammation, necrosis and miliary granulomas, focal leptomeningitis and localized chorioretinitis. As has been pointed out elsewhere,[67, 68] the resemblance of the pathological changes described in 3 infants by Jankû,[21] Torres,[59] and Richter [49] to those in our 1st patient makes it evident that these cases are instances of the same disease.

The lesions in each of the 5 affected infants contain a protozoan parasite showing the same morphological characteristics. In sections the microorganisms are oval, ovoid or pyriform, 2–3μ long and 1–2μ wide, and contain deeply staining chromatin bodies which are usually spherical and polar in position. This microorganism, as seen in our 1st case, was considered to be an Encephalitozoon because of its resemblance to that parasite in sections and because the characteristic lesion that the latter produces in the central nervous system is a miliary granuloma similar to that found in this human disease. Encephalitozoon corresponds in size and shape to the causative parasite of this disease, is frequently intracellular, and occurs regularly in the form of similar cysts. Although Torres [59, 60, 61] favored the identification of his microorganism as an Encephalitozoon and named it *Encephalitozoon chagasi*, he also entertained the possibility that it might be a Toxoplasma. Later, Levaditi,[26] in discussing the identity of

the parasites in the cases of Jankû and Torres, pointed out their similarity to Encephalitozoon and to *Toxoplasma cuniculi*. He referred to resemblances in the pathology of these cases to experimental toxoplasmic infection in rabbits, and favored the identification of the microorganisms in the human cases with Toxoplasma. It was noted in our first report that the lesions in the central nervous system show a resemblance to those in experimental toxoplasmosis in the rabbit: diffuse necrotizing and inflammatory lesions of the cortex and basal ganglia at times associated with miliary granulomas which do not become necrotic centrally and which are disseminated widely throughout the brain and spinal cord. This was discounted, however, for two reasons: (1) the microorganism of the human disease, as seen in sections, did not have the morphological characteristics commonly described for Toxoplasma — lunate shape, larger size, 4–6μ by 2–3μ, and central chromatin body; and (2) since the human infection was not transmitted to animals, an experimental infection produced by it could not be directly compared with experimental toxoplasmosis. During a restudy [68] of Richter's case in which a protozoon similar to that in our 1st case was identified, an opportunity arose to make some observations on the morphology and biological characteristics of a strain of Toxoplasma recovered from a guinea pig at the Rockefeller Institute. It was found that in sections of lesions of the brain and other organs in the rabbit and mouse, this Toxoplasma was almost identical in appearance with the parasite in our 1st case and in Richter's case. In smears, however, it had the more commonly described appearance of Toxoplasma given above. It became clear that apparent morphological differences between Toxoplasma and Encephalitozoon were insufficient to distinguish between them in sections and certainly could not serve as a basis for identifying an unknown parasite as one or the other. In retrospect the staining properties of the microorganisms might have furnished a minor clue to the identity of the protozoon of the human disease. It is stained easily with all the common stains and this is true of Toxoplasma of animal origin. Encephalitozoon, on the other hand, is stained by the carbol fuchsin stain and is less readily brought out by other common stains. Further, some features of the pathological lesions in the human disease, as noted above, are more like those

encountered in experimental toxoplasmosis in the rabbit than spontaneous Encephalitozoon infection in that animal. The granulomas in the central nervous system in Encephalitozoon infection often show central necrosis, while in this human disease and animal toxoplasmosis they do not. No diffuse inflammatory and necrotizing lesions are present in the brain in Encephalitozoon infection, whereas they are prominent in the human disease and occur in experimental toxoplasmosis. Spontaneous Encephalitozoon * infection is chronic, and in the majority of instances non-lethal, while the human disease and experimental toxoplasmosis, as a rule, are subacute and fatal. In the 1 case of the human disease in which other organs were involved, the heart showed lesions and the kidneys did not. This is more like toxoplasmosis than Encephalitozoon infection. Some of these points were discussed in the report of new observations in Richter's case, and it was pointed out there that the possibility that the human infection was toxoplasmosis rather than Encephalitozoon infection would have to be seriously considered. It was obvious, however, that until the human infection could be transmitted to animals and the microorganism and the experimental infection produced by it studied and compared with toxoplasmosis, no decision could be made.

The transmission of the infection from the infant (present case) to animals and the experimental studies [69] which followed yielded definite evidence that the causative microorganism in this case is a Toxoplasma. (1) The morphology of the microorganism isolated from the human case corresponded to that of Toxoplasma of animal origin. (2) The course of the disease and the lesions produced in the animals inoculated with it were similar to those noted in the same species by inoculation of a Toxoplasma of animal origin. (3) The susceptibility of the rabbit, mouse, guinea pig and chick to this microorganism corresponded to the wide host range of Toxoplasma of animal origin. (4) Convincing evidence

* The meager knowledge of Encephalitozoon is due to the fact that so few have been able to transmit the infection experimentally (and then with equivocal results [27]), and that it has not been possible to cultivate the microorganism. The resemblances between it and Toxoplasma make one wonder whether there may not be some close relationship between the two microorganisms. Our failure to transmit an Encephalitozoon infection has prevented us from directly comparing the biological characteristics of this parasite with those of Toxoplasma. It is remarkable that others who have worked with one or both of these organisms have not troubled to make any exact and clarifying comparison between them.

of the nature of the microorganism was obtained by cross-immunity experiments. Rabbits and mice immune to the human strain proved to be immune to the Rockefeller Institute guinea pig strain of Toxoplasma and *vice versa*. This experimental material will be described in detail in a forthcoming report. Although experimental evidence is lacking to establish the identity of the protozoa in the other 4 cases (our Case 1, and those of Jankû, Torres and Richter), their morphological resemblance to this proved Toxoplasma and the similarity of the lesions produced make it extremely probable that they, too, are Toxoplasmata. The disease represented by these 5 cases might, therefore, be designated toxoplasmic encephalomyelitis and the causative microorganism, *Toxoplasma hominis*.

Three cases of obscure human infections which have at times been cited as possible instances of toxoplasmosis do not withstand critical analysis. The 1st case was reported by Castellani [10] (1914) from Ceylon in a boy of 14 who had a prolonged fever, splenomegaly and severe anemia, and was markedly emaciated at death. The organs were described as normal grossly except for the spleen which was greatly enlarged. The nervous system was apparently not examined. No histological examination was reported. Smears of the blood and spleen revealed bodies that Castellani decided were Toxoplasmata and named *Toxoplasma pyrogenes*. There were two types, the larger of which measured 7–12μ in maximum diameter. They were rounded or pear shaped and contained several large masses of chromatin. The smaller measured 2.5–6μ in maximum diameter, were round, oval or crescentic in shape, and had one large rounded mass of chromatin at one pole or centrally placed. Wenyon [65] in discussing this case voiced a justifiable doubt as to the nature of the structures described. The larger forms he believed are portions of degenerated cells and the smaller forms probably vegetable organisms, like yeasts. The lack of any description of lesions in the organs which might be compared with those in animal toxoplasmosis renders any decision as to whether this was a toxoplasma infection or not even more difficult.

Fedorovitch [16] (1916) described a case of chronic fever in a boy of 10 years from the Black Sea district. This child also had anemia and a markedly enlarged spleen. Blood smears revealed

an organism which the author considered to be very much like the bodies described by Castellani. These were not present in material from a puncture of the spleen. The fate of the patient is not known and there is, therefore, no clue as to what pathological lesions may have been present. Wenyon [65] was of the opinion that the bodies were vegetable organisms like large cocci or yeasts.

Chalmers and Kamar [11] (1920) observed soldiers in the Sudan with chronic fever, headache, slight cough and diarrhea, and terminal severe anemia and gingivitis. The pathological lesions in an autopsy performed on 1 individual were not recorded except for a photograph of a splenic film. This illustrates bodies which the authors do not describe but state are similar to those Castellani considered to be Toxoplasmata. Wenyon [65] believed these bodies to have been altered Leishmania.

It is clearly impossible to determine whether or not these are cases of human toxoplasmosis. The pathological lesions are not described so that a comparison cannot be made with the lesions in animal toxoplasmosis, and transmission to animals was not attempted for the purpose of identifying the organisms. Although the morphology of some of the single bodies may suggest Toxoplasma, cysts are not described and some of the structures illustrated are quite unlike Toxoplasma.

That there may be forms of human toxoplasmosis other than granulomatous encephalomyelitis of infants is possible. One cannot, however, accept these 3 cases as instances of another type of toxoplasmosis in the face of the inadequacy of the data concerning them.

From a review of the 5 cases of toxoplasmic encephalomyelitis, the disease is found to have the following characteristics:

Clinical Features: Although the records are not always complete, the clinical data in this series of patients are sufficiently alike to furnish at least the outlines of a syndrome (Table I). The patients were all infants, 4 of whom died before the age of 2 months. The youngest of these was 2 days of age and the oldest 7 weeks, the duration of the illness having varied from 2 to 28 days. The age at death in the 5th case, that of Jankû, was not clear from the record, but appears to have been the 11th or 16th month, and the duration of the illness was not exactly stated, but

TABLE I

Summary of Clinical Data in Five Cases of Toxoplasmic Encephalomyelitis

Case	Location	Sex	Age at death	Duration of illness	Symptoms and signs	Ophthalmoscopic examination	Spinal fluid
Authors' 1st case J. S.[57]	New York City	F	30 days	28 days	Convulsions, diarrhea, vomiting, labile body temperature, slight enlargement of head (?), disturbances in respiration	Yellowish white focal area of chorioretinitis, bilateral	Xanthochromia, high protein content, pleocytosis (largely mononuclears), 2 eosinophils in smears
Jankû [21]	Prague, Czechoslovakia	M	11–16 mos. (?)	8–11 mos. (?)	Marked hydrocephalus, blindness, convulsions, anorexia, vomiting	Yellowish white focal areas of chorioretinitis bordered by pigment in macular region bilaterally	
Torres [59, 60, 61]	Rio de Janeiro	F	2 days	2 days (?)	Convulsions		
Richter [49]	Chicago, Ill.	F	7 weeks	1 week (?)	Convulsions, opisthotonos, fever, "cold"		Xanthochromia, high protein content, pleocytosis (mild, cell type not stated)
Authors' 2nd case C. D.	New York City	M	31 days	28 days	Convulsions, vomiting, sensory and motor signs of cervical cord lesions with subarachnoid block, labile body temperature, disturbances in respiration	Reddish brown areas of chorioretinitis in macular regions bilaterally	Cloudy xanthochromic fluid, high protein content, clotted spontaneously (no microscopic examination)

seems to have been at least 8 months. Two of the infants were males and 3 were females.* All suffered convulsions at some time or other during their illness. Two had enlargement of the head due to internal hydrocephalus. In 3 of the infants there was a labile body temperature with occasional mild fever or subnormal temperature. In 3, vomiting occurred at intervals. The spinal fluid from 3 of the patients was examined and showed xanthochromia and a high protein content, and in 2 there was pleocytosis. The spinal fluid smear in 1 case revealed occasional eosinophils in addition to mononuclear cells. In this case parasites were identified in paraffin sections of precipitated ventricular fluid. An ophthalmoscopic examination was made of 3 of the infants and in all a focal chorioretinal lesion was found in each eye. The disease, then, appears to affect young infants, produces manifestations of involvement of the nervous system, may give rise to ophthalmoscopically identifiable focal lesions in the eyegrounds, and terminates fatally after an acute or subacute course.

Gross Pathology: The pathological picture of the disease is distinctive. The central nervous system is the site of the most severe and widespread changes. Macroscopically depressed, softened, yellowish focal lesions varying in size from a few millimeters to 3 cm. in diameter are seen on the surface of the cerebrum. In these areas the convolutional markings are obliterated and the overlying leptomeninges are thickened and rendered opaque by a grayish yellow exudate. There may be swelling of the gyri at the margins of these lesions while the intervening gyri and their overlying leptomeninges appear normal, except for a varying amount of congestion. The spinal cord may show similar focal areas of softening and collapse, or considerable localized swelling with an accompanying leptomeningeal reaction limited to such regions.

The cerebral lesions are often confined to the cortex but may involve the subcortical white matter and centrum ovale and reach the wall of the lateral ventricle. They are well demarcated and range in size from minute nodules to patches several cm. in diameter. The center of such lesions is yellowish, soft, and sometimes cheesy, while the edges may be grayish and glassy. In some

* Torres' patient, whose sex is not given in the author's report, was a female (personal communication).

of the cerebral foci gritty material may be palpable. Others may contain cysts which occasionally reach a diameter of several cm. Focal changes similar to those in the cortex and white matter are encountered in the corpus striatum, thalamus and hypothalamus, and may occur in the midbrain, pons and medulla as well. The cerebellum shows minor lesions in 1 case. There may be a varying degree of enlargement of the lateral and third ventricles due in part to stenosis of the aqueduct of Sylvius, and partly to degeneration of the ventricular walls. The latter may be a prominent gross lesion and is seen as a well demarcated broad band of yellowish, soft, friable periventricular necrotic tissue. It is associated with extensive denudation of the ependymal lining. Coarse ependymal granulations may be present in the better preserved portions of the ventricular walls. The lesions in the spinal cord may also vary from minute yellow foci to large confluent softenings involving the entire diameter of the cord in a number of segments, obliterating all the normal architectural markings, and leaving only yellow pultaceous material.

A complete autopsy was performed in 3 of the 5 cases: Torres', Richter's, and our 1st case. The last 2 cases showed no pertinent gross changes in any of the viscera, bones or muscles. Torres gave no gross description of his material except for a brief reference to the brain, although microscopic changes were referred to in some of the other organs, as will be noted below.

Microscopic Examination: The histological changes are characteristic. There is a widely disseminated encephalomyelitis in which the severest lesions are associated with necrosis. There is a total destruction of neural and neuroglial elements in the areas of degeneration leaving amorphous débris. In the marginal necrotic material, lipoid laden phagocytes and often polymorphonuclear leukocytes are present. There is a bordering zone of reaction containing hyperplastic capillaries, plasma cells, lymphocytes, mononuclear leukocytes, neutrophils and eosinophils. Fibroblasts derived from hyperplastic capillaries and from the contiguous leptomeninges often mingle with these cells, and in places where the capillary proliferation is intense, give rise to a rich granulation tissue in which many collagen and reticulum fibers may occur. Beyond the zone of cellular infiltration there is a moderate astrocytosis as well as hypertrophy and multiplication of microglial cells

with production of rod cells and transitional phagocyte forms. Cavities may occur in the necrotic zones and contain persisting hyperplastic capillaries and varying numbers of lipoid laden phagocytes. Degenerated tissue may become partially or completely calcified and give rise to broad bands of calcific material in the cortex or periventricular zone. Incrustation of nerve cells and other elements occurs in these areas.

Miliary granulomas constitute a prominent feature of the pathological process in the nervous system. They are found scattered through the brain and spinal cord and often cluster near the focal necrotic and inflammatory lesions. Each granuloma is composed of a circumscribed group of closely approximated epithelioid cells, evidently of capillary endothelial origin. Mingled with these, usually in the outer portion of the granuloma, are varying numbers of plasma cells, lymphocytes, mononuclear leukocytes and eosinophils.

The leptomeninges overlying the parenchymal lesions show a moderate hyperplasia of their cells with the production of fibroblasts, and they are infiltrated by cells similar to those seen in the parenchymal exudate. Here again plasma cells and eosinophils are a characteristic feature of the inflammation. Often the exudate is continuous from pia-arachnoid to parenchyma, obliterating the line of demarcation between them. Many of the leptomeningeal capillaries in these areas of inflammation and some of the larger vessels show endothelial hyperplasia. There is no occlusion of leptomeningeal or parenchymal vessels to account for the areas of necrosis, although secondary thrombosis is encountered. Infiltration of the connective tissue core of the choroid tufts and tela choroidea may be present. The walls of the lateral and third ventricles are frequently denuded of ependyma and may be covered by exudate. Where the ependyma is intact there are at times numerous ependymal granulations and subependymal gliosis. This may lead to stenosis of the aqueduct of Sylvius.

The focal lesions in the eyes consist of patches of severe chorioretinitis. The involved retina is swollen and shows varying degrees of degeneration. Total necrosis may occur in some areas and involve many of the layers. The internal limiting membrane is at times disrupted. At the margins of completely necrotic areas the cells of the ganglionic layer are degenerated or disappear.

The deeper layers, although edematous, are better preserved except for the layer of rods and cones and the pigmented layer, which are often necrotic and disrupted. Free pigment granules as well as dislocated pigmented cells are often extruded into the internal retinal layers. There may be scattered plasma cells and lymphocytes in the necrotic areas with moderate infiltration by similar cells as well as occasional eosinophils, neutrophils, mononuclear leukocytes and lipoid laden phagocytes in the marginal zone. In the more severely affected layers of the retina there is an increase in glial tissue and formation of granulation tissue. The latter may invade the posterior chamber. An exudate similar to that described above may be present on the internal surface of the retina. The choroid is congested, hyperplastic, and infiltrated chiefly by plasma cells and lymphocytes. A mild similar infiltration may occur in the sheath of the optic nerve. The sclera remains unchanged.

Inflammatory lesions may occur in other organs, as reported in Torres' case. This author mentions the presence of "disseminated foci of myositis" in the striated muscles, and "acute diffuse myocarditis" in which the predominant cells are mononuclear in type with an admixture of numerous eosinophils. Complete autopsies in 2 other cases revealed no lesions in the other organs except for a terminal bronchopneumonia in 1.

Microorganisms: A comparison of the morphology of the parasites in the 5 cases referred to in this paper makes it seem probable that we are dealing with the same organism, or at least closely related forms in each instance. The parasites seen in sections of fixed tissue, using any of the common stains, are usually ovoid corpuscles with a spherical polar chromatin body. They may be oval, pyriform, rounded or occasionally lunate, and the chromatin body may rarely be centrally placed and band-like. They also occur less frequently in clusters which have been described as cysts. The cysts are composed of varying numbers of closely packed organisms which for the most part are clearly outlined but sometimes seem to form a syncytial mass. A cyst wall appears to be present but this may be the margin of a matrix in which the parasites are embedded. The apparent cyst wall, on the other hand, may be the remains of the cytoplasm of a parasitized cell.

Parasites are present in small numbers in the leptomeningeal exudate. Large numbers are found in the inflammatory tissue at the margins of the necrotic parenchymal lesions, and numerous degenerated forms are present in the central débris. Moderate numbers of parasites occur in the granulomas. They are abundant in the focal chorioretinal lesions. In the 1 case (Torres) in which other organs were involved, namely the heart and striated muscles, parasites were seen in the lesions and microorganisms were also described in the subcutaneous tissue.

The parasites may be free or intracellular. The intracellular forms occur in large mononuclear cells and epithelioid cells of granulomas, less frequently in polymorphonuclear leukocytes, and rarely in the endothelial cells of the capillary walls, eosinophils or nerve cells. It is probable that they occur in other cells such as ependymal, choroidal and leptomeningeal elements, but as yet they have not been found parasitizing such cells.

Some minor differences in the size and morphology of the parasites in the 5 cases do not appear to be significant. The majority of the single organisms as described above in paraffin sections of formalin and Zenker-fixed tissue measure 2–3μ in length and 1.5–2μ in width. Torres finds the length of the individual organisms to be slightly greater (3.5μ), some of those in the muscles reaching a length of 6μ. In Richter's material the parasites measured 2–2.5μ in length and 1.5–2μ in width, figures slightly smaller than those given in our cases. All of these measurements are average figures and do not indicate the overlapping which occurs when the extremes are included. In any case the differences in fixation and in preparation of the tissues may well account for the slight dissimilarities noted. The variation in the size of the cysts, particularly the larger average sizes given by Jankû, probably depend on similar factors and do not appear to be important. The apparent differences between the appearance of the microorganisms in these human cases and that of Toxoplasma, as generally described in the literature, would seem to depend on differences in histological technic. As has been demonstrated, Toxoplasmata from an animal source, when seen in sections of embedded tissue, are exactly like those in the human cases, while the microorganisms in smears assume the larger lunate form commonly described for Toxoplasma.

Mode and Source of Infection: In considering the mode of infection in 3 of these cases, in a previous paper it was pointed out that the onset of the clinical manifestations of the disease soon after birth in 2 cases, and the advanced nature of the lesions, suggested that the infection had begun during intrauterine life. This is supported by the evidence derived from the case reported here in which symptoms began on the 3rd day of life. Although blindness was first noted in Jankû's patient at 3 months of age, Jankû considered the infection congenital since a maldevelopment of one eye was present which he thought ascribable to infection during fetal life. In Richter's case the infant was said to have become ill at the age of 6 weeks, but the incompleteness of the clinical data permits a doubt as to whether the onset was not at an earlier age. It was Richter's opinion that the infection was congenital in view of the apparently advanced age of some of the pathological changes. As in the case described here, there was marked calcification of many of the focal lesions.

That the mothers of the 5 infants were apparently in good health does not preclude the possibility of the infection having occurred *in utero*. It is conceivable that they were carriers of a clinically inapparent Toxoplasma infection to which the fetus was more susceptible. Toxoplasma have often been found in animals which showed no clinical symptoms, although the virulence of the parasites has been demonstrated by their ability to produce active infection when inoculated into other animals. If the infection was transmitted *in utero*, it might be expected that the placenta would show specific pathological changes. Unfortunately the placentas were not examined in any of these cases. It is to be hoped that in the future such an examination will be made. How the mothers might have been infected, or the children, if they acquired the infection independently after birth, is not clear.

As mentioned above, Toxoplasma has a widespread geographic distribution, and pathogenicity for a wide variety of hosts, but its natural mode of transmission is as yet unknown. Experimentally toxoplasmosis has been successfully transmitted by inoculation of infected material by many routes.[28] Infection has followed cannibalization [51] of animals recently dead of the disease. Instillation of infected peritoneal fluid into various cavities lined by mucous

membrane, including the vagina and conjunctival sac, has resulted in a generalized infection in some instances.[36]

As has been noted previously, Jankû, apparently unaware of the occurrence of spontaneous Toxoplasma infection in rabbits, alluded to the fact that the mother of the infant he described had been resident during her pregnancy on a farm where rabbits were raised, and that she had eaten much rabbit meat during the period of gestation. In Torres' and Richter's reports no reference was made to contact of the mother with animals. It is of interest in relation to Torres' case that Brazilian rabbits and birds are known to be infected with Toxoplasma. In our 1st case the home was so overrun with mice that the mother changed her residence in the midst of her pregnancy. Although spontaneous toxoplasmosis has not been described in mice in North America, its occurrence in that species is known in other parts of the world. There was also a canary in the home during the pregnancy in our 1st case. These birds have been found to be subject to toxoplasma infection. In the case reported here there was no history of contact with animals.

In the 2 instances in which there was contact of the mother with animal species which may harbor Toxoplasma there is a possibility that transmission occurred by ingestion of infected material, *i.e.*, rabbit meat in Jankû's case, and food contaminated by mouse excreta in our 1st case. The existence of an insect or other vector as an intermediate host between a possible animal carrier and man cannot be excluded. The portal of entry of Toxoplasma in the mother and its localization prior to infection of the infant remain undetermined in the light of our present knowledge. In view of the report of experimental vaginal infection with Toxoplasma one must consider the possibility of vaginal infection in the mothers with direct extension of the infection to the uterine contents. This hypothesis seems unlikely.

The possibility that a maternal vaginal infection was transmitted to the infant in its passage through the birth canal is excluded in our 2nd case in view of the fact that delivery was by Cesarean section, and by analogy is unlikely in the other instances.

If the infection be congenital it is probable that it occurs late in fetal life since developmental anomalies were absent except in 1 instance (malformation of the eye in Jankû's case). It is clear that

no absolute evidence exists that the infants became infected before birth. This problem has yet to be attacked experimentally in animals and evidence sought in future human cases.

Pathogenesis: The description of the probable mode of spread of the infection, the susceptibility of various organs and tissues, and the development of the lesions, as summarized in the discussion of our 1st case, apply to the case reported here as well. No new facts as to the pathogenesis of the disease have been added by a study of the pathological material from the present case. Study of the characteristics of the infection in animals has afforded an opportunity to test these hypotheses of the pathogenesis of the human disease and they will be discussed in the presentation of the experimental material.

Diagnosis: Although it may be premature to attempt to establish criteria for the clinical diagnosis of toxoplasmic encephalomyelitis, it is useful to summarize the facts available to date which may lead to a recognition of the disease during life. Young infants of either sex become ill soon after birth. They are subject to repeated convulsions and may show symptoms and signs of widespread involvement of the central nervous system. Ophthalmoscopically, focal areas of chorioretinitis are seen in each eye. These appear as yellowish white or brownish red, round or oval patches which may show irregular black, often marginal pigmentation. The intense calcification of the cerebral lesions in some of these cases suggests that stereoroentgenograms of the skull may reveal the presence of such changes. The cerebrospinal fluid is xanthochromic, contains a large amount of protein, and shows a pleocytosis chiefly of round cells. Eosinophils have been found in ventricular fluid. Toxoplasmata have been identified in fluid from the same source and it seems probable that intracerebral inoculation of such fluid into rabbits or mice will demonstrate the presence of the parasite by the production of a specific infection.

Summary and Conclusions

1. A 5th case of a new disease, granulomatous encephalomyelitis due to a protozoon, occurring in an infant is described.

2. The clinical and pathological observations in this case are shown to be similar to those in the first 4 cases. This group forms a distinct disease entity. The disease affects young infants, pro-

duces manifestations of generalized involvement of the nervous system, may give rise to ophthalmoscopically identifiable focal lesions in the eyegrounds, and terminates fatally after an acute or subacute course. The spinal fluid shows xanthochromia, a high protein content and pleocytosis. The central nervous system is the site of focal inflammatory and degenerative lesions which are widely disseminated. Similar changes are found in the retina and choroid. Miliary granulomas are a characteristic feature of the process in the nervous system. Focal inflammatory lesions were present in the heart and striated muscle in 1 case.

3. A protozoan parasite is present in all the lesions.

4. The results of transmission of the infection to animals from the case reported here indicate that the causative protozoon is a Toxoplasma. The designation *Toxoplasma hominis* is suggested for the microorganism and the term toxoplasmic encephalomyelitis for the disease.

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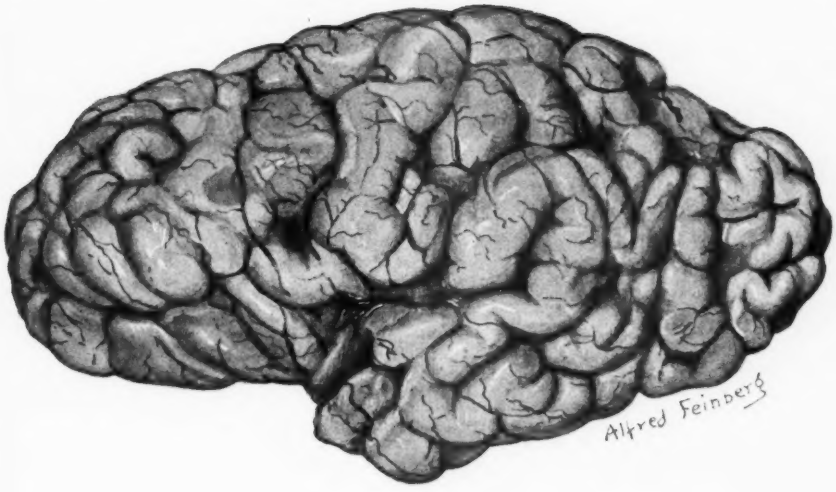
DESCRIPTION OF PLATES

PLATE 99

FIG. 1. Left cerebral hemisphere showing depressed, yellowish and softened cortical lesions.



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Wolf, Cowen and Paige Toxoplasmic Encephalomyelitis

PLATE 100

FIG. 2. Left cerebral hemisphere, coronal section. Focal necrotizing lesions are present in the cortex and in the subcortical white matter.

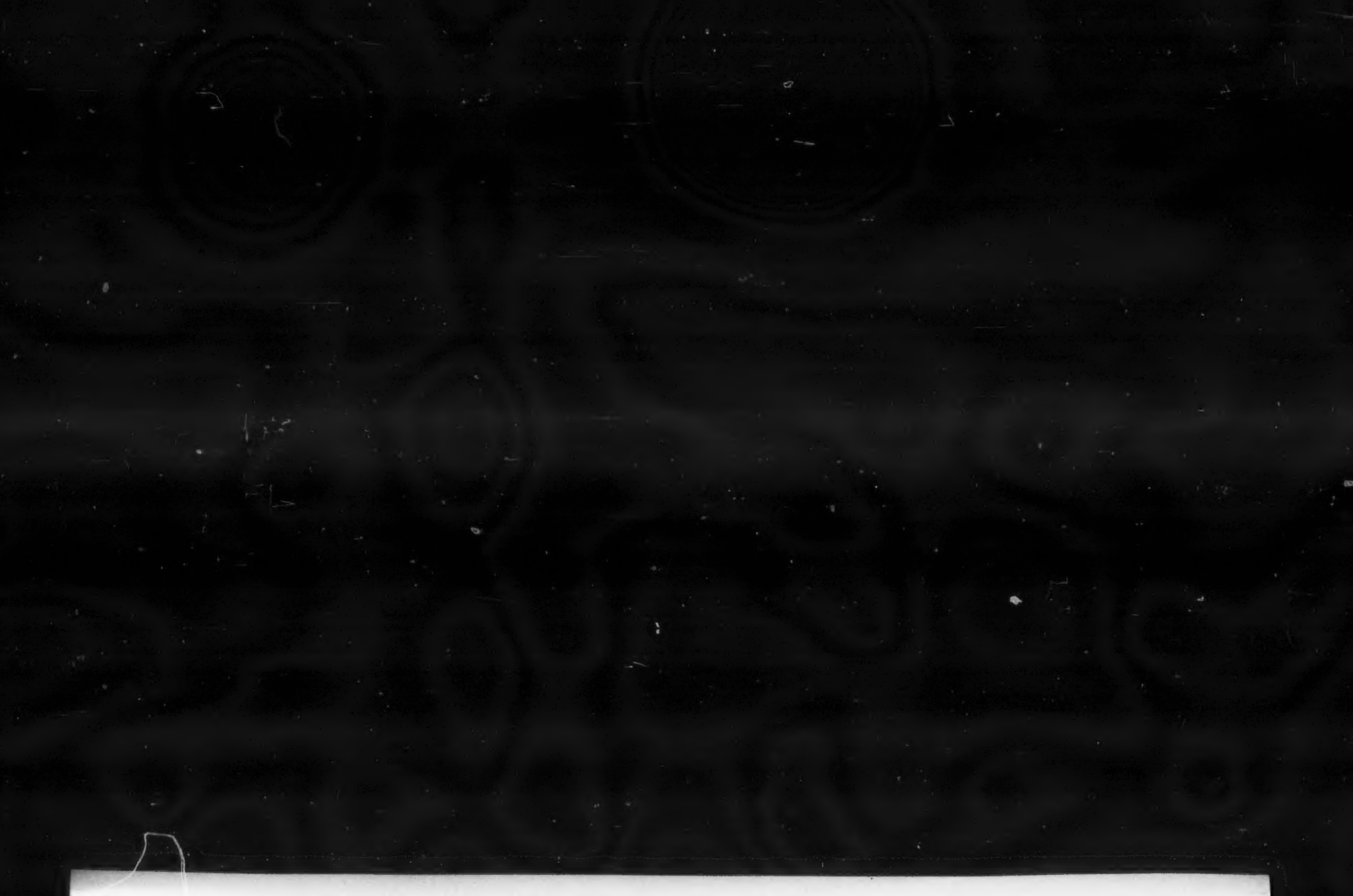
FIG. 3. Cervical spinal cord showing swelling of cord and obliteration of normal architectural markings.

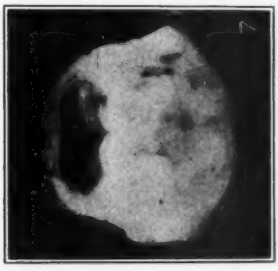
FIG. 4. Upper thoracic spinal cord showing discoloration with obscuration of normal markings.

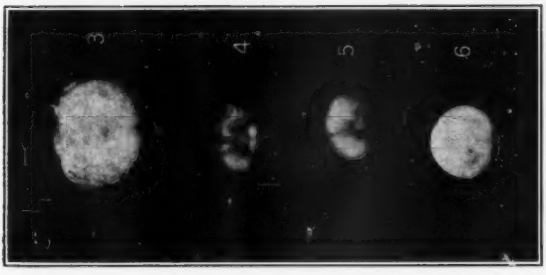
FIG. 5. Lower thoracic spinal cord showing changes similar to those present in Fig. 4.

FIG. 6. Lumbar spinal cord showing obliteration of posterior columns and horns by grayish tissue.

FIG. 7. Pons. A sharply demarcated focal lesion is present at the junction of the tegmentum and the reticular zone.







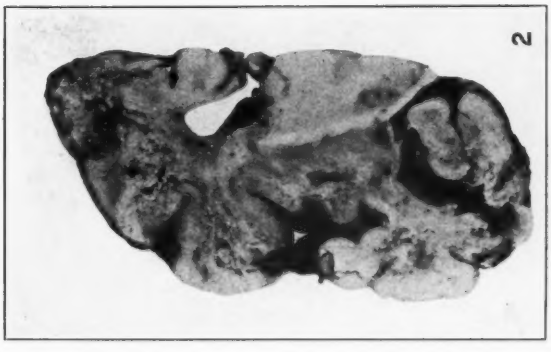


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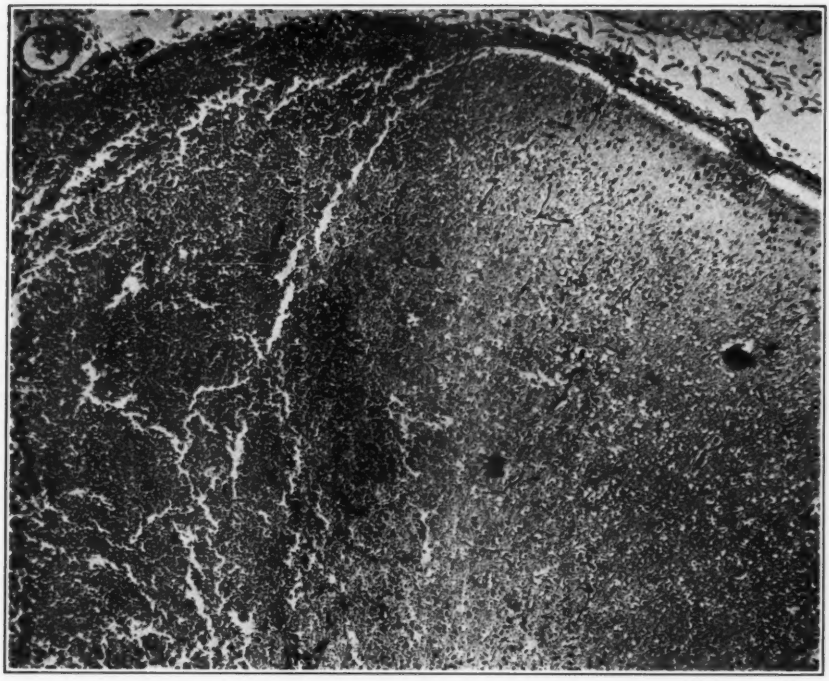
PLATE 101

FIG. 8. Cerebral cortex. A focal inflammatory and necrotizing lesion is present in the cortex. Note the comparatively sharp margin and accompanying focal leptomeningitis. The adjacent cortex on the right is relatively well preserved. Hematoxylin-eosin stain. × 80.

FIG. 9. Cerebral cortex. Note the marked calcification of a necrotizing cortical lesion. Hematoxylin-eosin stain. × 80.



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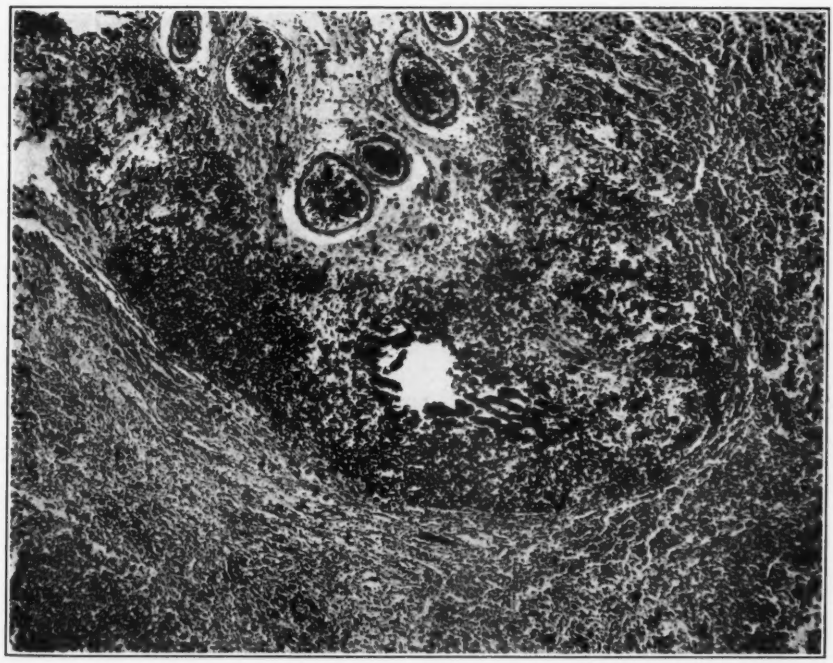

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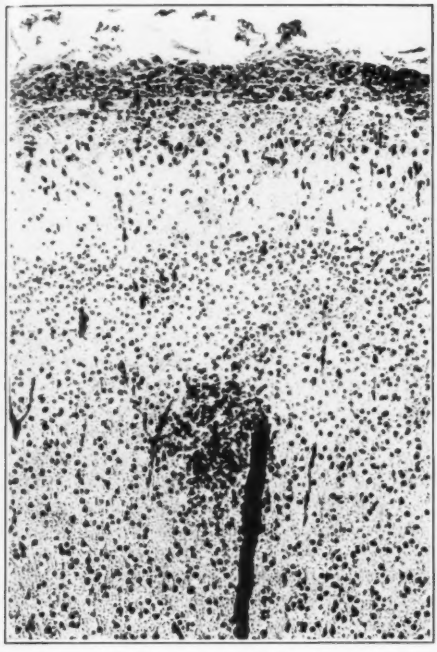
PLATE 102

FIG. 10. Cerebral cortex. A granuloma is present in the third cortical layer adjacent to a blood vessel. Focal leptomeningitis. Hematoxylin-eosin stain. × 150.

FIG. 11. Cerebral leptomeninges. Focal leptomeningitis. Lymphocytes, plasma cells and large mononuclear leukocytes are present in the exudate. Lipoid laden phagocytes, lymphocytes and plasma cells are seen in the underlying degenerated cortex. Hematoxylin-eosin stain. × 300.

FIG. 12. Lumbar spinal cord. White matter showing a granuloma composed of epithelioid cells and a few lymphocytes. Hematoxylin-eosin stain. × 300.

FIG. 13. Wall of the lateral ventricle. Inflammation, necrosis and loss of the ependymal lining are present. Hematoxylin-eosin stain. × 150.



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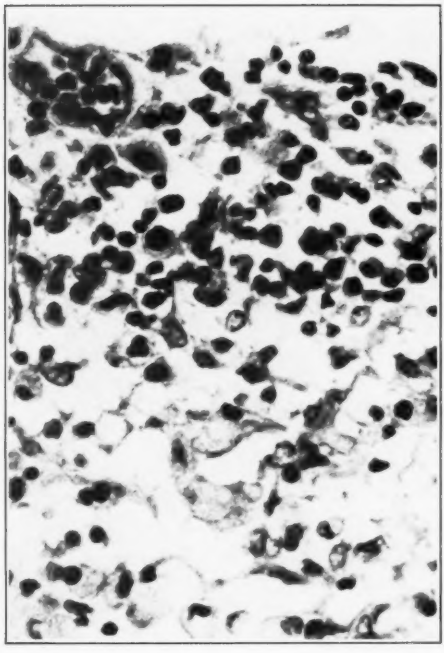

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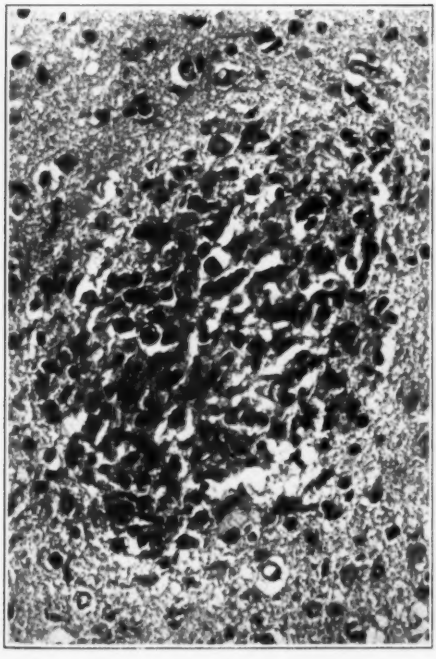

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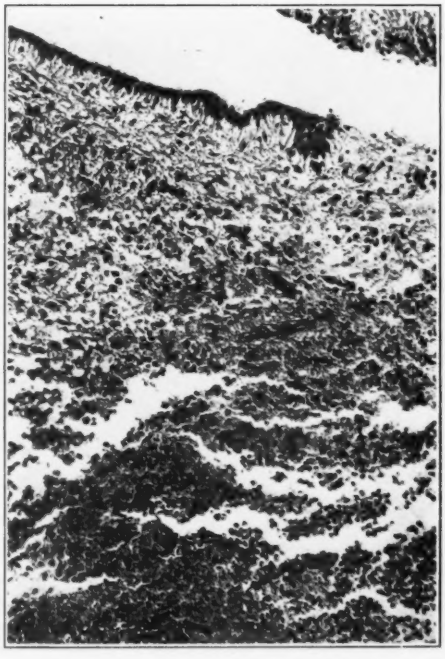

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Wolf, Cowen and Paige Toxoplasmic Encephalomyelitis

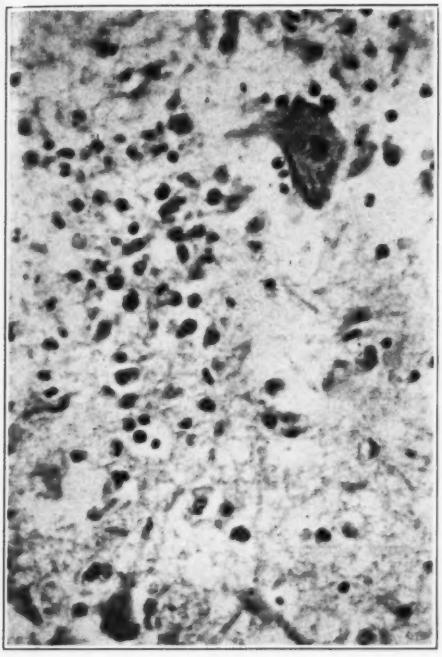
PLATE 103

FIG. 14. Cervical cord. Section of anterior horn showing an acute inflammatory and necrotizing lesion with infiltration by polymorphonuclear leukocytes, lymphocytes and large mononuclear cells. Hematoxylin-eosin stain. × 250.

FIG. 15. Cerebral cortex. Calcification is seen in a degenerated focal lesion in the cortex. Note the encrusted nerve cells. Hematoxylin-eosin stain. × 200.

FIG. 16. Granuloma with surrounding astrocytosis. The epithelioid cells in the granuloma are obviously unrelated to astrocytes. Cajal's gold sublimate stain. × 300.

FIG. 17. Lumbar spinal cord. Miliary granulomas in the lateral and the anterior white columns. Giemsa's stain. × 150.



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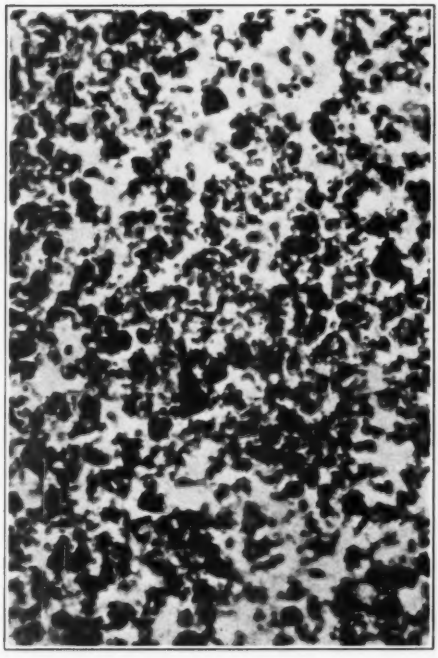

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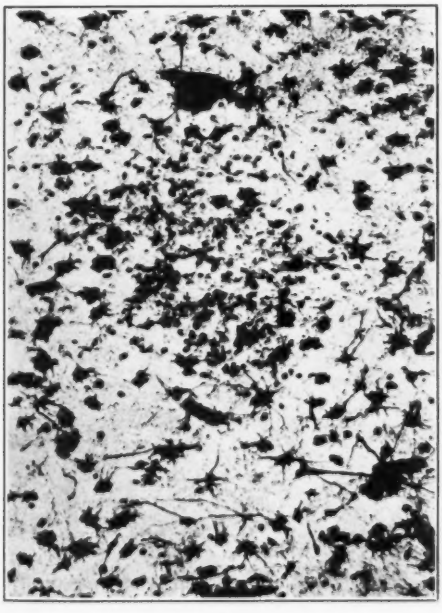

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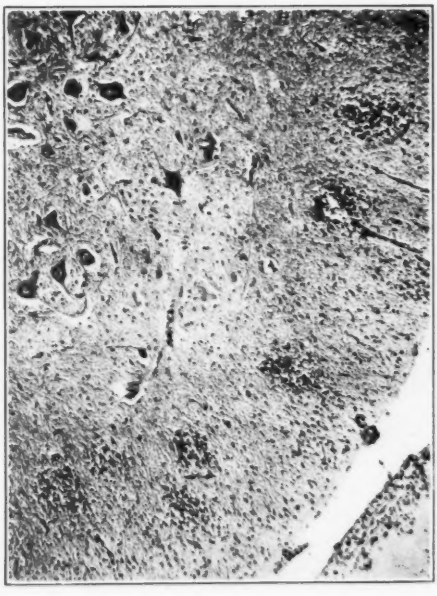

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Wolf, Cowen and Paige

Toxoplasmic Encephalomyelitis

Plate 104

Fig. 18. Lumbar cord. Note the intense necrosis of the posterior and portions of the lateral columns, the posterior and parts of the anterior horns and the central gray matter and commissures. Pal-Weigert stain. × 20.

Fig. 19. Retina of the right eye. Note the edema and degeneration of the retina and infiltration of the choroid. Hematoxylin-eosin stain. × 40.

Figs. 20–27. Show parasites in lesions in the cervical cord and pons. × 1050.

Fig. 20. Single oval intracellular parasite with polar chromatin body. Phloxine-hematoxylin stain.

Fig. 21. Two free ovoid parasites. Hematoxylin-eosin stain.

Fig. 22. Four free oval parasites. Hematoxylin-eosin stain.

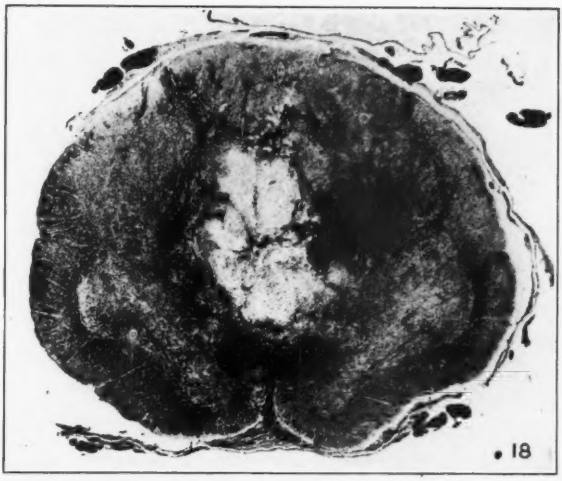
Fig. 23. Three intracellular round and oval parasites. Hematoxylin-eosin stain.

Fig. 24. One round intracellular parasite showing division of its chromatin body. Hematoxylin-eosin stain.

Fig. 25. Two intracellular parasites, one binucleated, apparently dividing. Hematoxylin-eosin stain.

Fig. 26. Parasitic cyst. Hematoxylin-eosin stain.

Fig. 27. Parasitic cyst rupturing and liberating parasites. Hematoxylin-eosin stain.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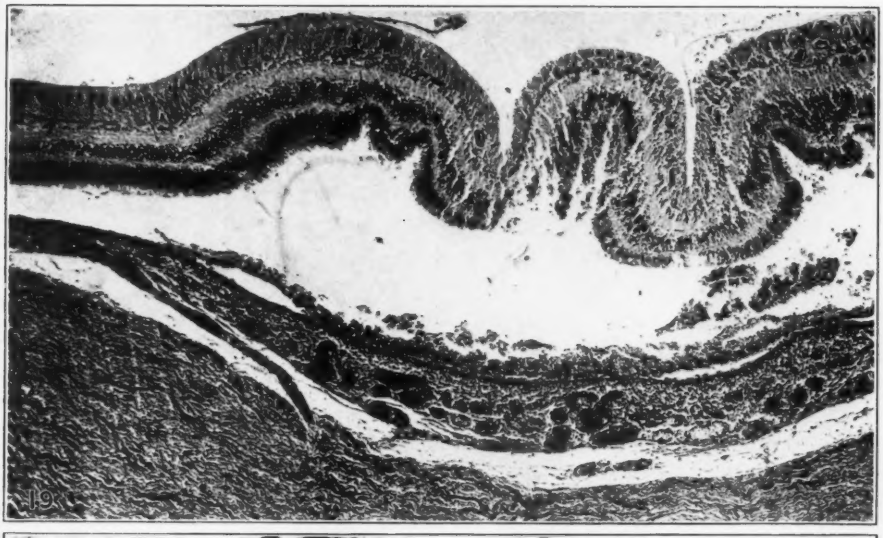
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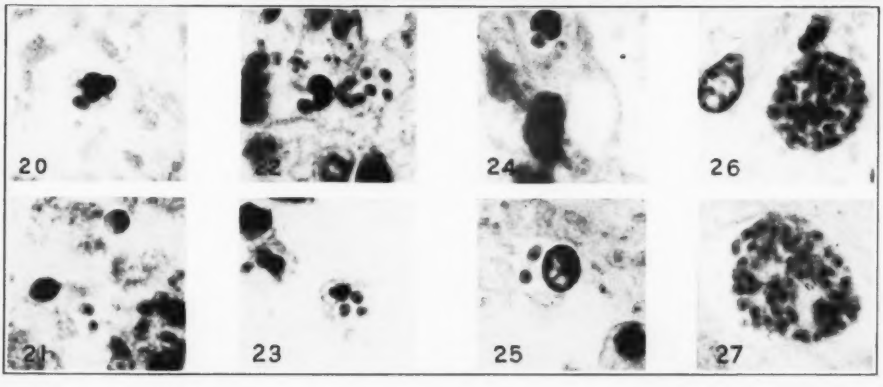
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EXPERIMENTAL PRODUCTION OF ENDOCARDITIS LENTA *

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Rosenbach [1] in 1878 passed an instrument down the right carotid artery in dogs and rabbits to wound the valves of the heart. Two of his dogs, apparently through unintentional infection, developed large vegetations on the aortic valves with septic infarcts and petechial hemorrhages in various organs. Colonies of organisms, apparently staphylococci, were demonstrated in these lesions. Ribbert [2] later succeeded in producing endocardial lesions without direct wounding of the valves by injecting into the ear vein of the rabbit a suspension of staphylococci from potato cultures so prepared as to include starch grains and larger fragments of potato. Large doses were required and in the successful experiments the animals died within 60 hours. Ribbert recognized that the disease was unlike human endocarditis. Dietrich [3] has more recently elaborated the experiments of Ribbert by introducing various substances parenterally to injure the valves of the heart and then injecting staphylococci or colon bacilli to cause the bacterial vegetations.

Dreschfeld [4] in 1887 appears to have been the first to transmit to experimental animals streptococci from a case of fatal human endocarditis, with resultant vegetations on the heart valves, which he accomplished by merely injecting the culture into the jugular vein of rabbits. It now seems probable that this investigator actually transmitted to rabbits the specific variety of endocarditis associated with the presence of *Streptococcus viridans*, although this organism was not clearly distinguished from other streptococci in 1887. Some 20 years later, Horder [5] injected 10 cc. of a broth culture of *Streptococcus salivarius* (*viridans*), isolated from the blood of a patient with chronic malignant endocarditis, intravenously into a rabbit weighing 2170 gm. The injection was repeated 10 days later. After a further interval of 38 days the rabbit died

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and at autopsy there were massive mitral vegetations projecting into the auricle and containing the same streptococcus. Similar results were obtained with streptococci isolated from normal human saliva and normal human feces. Photographs of the lesions are shown in his publication. Rosenow [6] in 1912 used streptococci of the viridans group isolated from the blood and from the tonsils of endocarditis patients and injected single large doses or repeated smaller doses intravenously into rabbits. Many of his animals succumbed in a few days but some of them survived for longer periods and in these he observed gross vegetations on the heart valves and in some the scars of healed valve lesions. Single very large doses or repeated smaller doses given intravenously to half grown rabbits proved to be the most successful procedure. Old rabbits were resistant and in young rabbits the lesions tended to heal quickly. Later Rosenow [7] used eight strains of *Streptococcus viridans* from cases of chronic septic endocarditis and produced lesions in 84 per cent of 44 rabbits injected. Apparently these animals were killed a day or two after inoculation and the small lesions were of somewhat uncertain significance for the endocarditis problem.

Experimental endocarditis produced by bacteria other than the *Streptococcus viridans* from human cases of endocarditis has been reported many times. Particularly significant is the report of Lloyd-Jones [8] who induced endocarditis in rabbits with great regularity by repeated daily intravenous injections of hemolytic streptococci, and particularly by such repeated injection of serum-broth cultures of viridans types of streptococci isolated from the human intestine and uterine cervix. More recently Blahd and her associates [9] injected a particular strain of beta-hemolytic streptococcus intravenously into dogs with production of endocardial vegetations in 40 per cent of the animals. There are also numerous reports of experimental endocarditis produced by mechanical wounding of the endocardium or by the introduction of gross foreign bodies in addition to the bacterial inoculation, recalling the pioneer observations of Rosenbach. Thus Welch and his associates [10] wounded the endocardium with a needle and resorted to accessory respiratory infection and dietary restriction in order to lower the resistance of their experimental animals. Friedman and his associates [11] anchored within the heart or great vessels a minute

Bakelite capsule enclosing a culture of the bacteria. Kinsella and Muether [12] passed a metal rod down the right carotid artery to wound the valves and subsequently introduced the bacteria by intravenous injection and by feeding. Such technical procedures are essentially different from the methods of Dreschfeld and of Horder.

Some of our earlier, less conclusive experiments may be briefly mentioned. For example, Rabbit 77 was injected intravenously with rather small doses of *Streptococcus viridans*, strain D, a suspension of approximately 5,000,000 bacterial cells daily from August 10 to Sept. 28, 1936; then 10,000,000 daily from September 29 to October 4; 15,000,000 daily from October 5 to 11, and 20,000,000 daily from October 12 to 29. Blood cultures taken from time to time just preceding the respective daily injection all remained sterile. The animal was sacrificed on Nov. 6, 1936, and the heart revealed no recognizable lesions. In 1937 we obtained our first positive result by injecting intravenously larger doses of living culture according to the technic of Lloyd-Jones. Rabbit 12 was inoculated with a culture of endocarditis strain M, 500,000,000 bacteria on Jan. 19, 1937, and increasingly larger doses on succeeding days up to 2.5 cc. of serum-broth culture on January 22, 23, 25 and 26, and 2 cc. on January 27. Blood cultures taken on January 23, 26 and 27 gave positive growth and the inoculations were discontinued. However, the blood cultures taken on January 28, 30 and February 2 remained negative. So after 12 days without inoculation the intravenous injections of culture were resumed, 2 cc. daily on February 9, 10, 11 and 12, and 1 cc. on February 13. The blood culture taken on February 12 became positive and hence the inoculations were again discontinued. Additional blood cultures taken on February 15 and 23 also gave a positive growth of the streptococcus. Without further treatment the rabbit died on March 4, 1937, and at autopsy there was found a gross vegetation on the mitral valve. Four other rabbits, 11, 19, 466 and 467, were inoculated by a similar technic with endocarditis streptococci, strains S, M, W and G, from different patients during the early months of 1937 without production of any convincing gross lesions on the heart valves. The experimental work was then interrupted.

During 1938 further experiments of this kind were undertaken

with strains of *Streptococcus viridans* obtained by blood culture from clinically typical endocarditis lenta. For our more successful inoculations the bacteria were grown in broth enriched with blood plasma or blood serum of the rabbit and the inoculations were made by injection into the ear vein of large doses of this whole culture, 1 to 6 cc., repeated daily for 6 days. After a pause of 48 hours, usually over Sunday, a blood culture was taken and then the series of 6 daily doses repeated. This program * was continued for variable periods until the evidence of successive positive blood cultures and progressive loss of weight made us believe that endocardial vegetations were established. The inoculations were then discontinued and the disease allowed to take its natural course or, in some instances, various therapeutic procedures were undertaken. During the subsequent course of the disease observations of the body weight and frequent blood cultures were made. Autopsy was performed as soon as possible on all animals that died. A preliminary note in regard to 21 of these animals was presented at the March meeting of the Society for Experimental Biology and Medicine [13] and some of the specimens from the animals were shown in the exhibit of the International Museums Association at Richmond, Virginia, April 5, 1939.

For many of these more recent experiments we used endocarditis strain P, *Streptococcus viridans*, obtained repeatedly in pure culture from the blood of a patient with endocarditis lenta. The organism ferments dextrose, saccharose, maltose and inulin, but not lactose, salicin, mannite or raffinose. It is not very toxic. We have been able to inject intravenously into volunteer donors as much as 1,500,000,000 heat-killed bacteria of this strain without recognizable reaction. Ten rabbits were inoculated with this bacterial strain, beginning on Dec. 5, 1938. Nine of these died and were examined sufficiently for present use. The 10th animal has, as yet, been inadequately studied.

Rabbit 458, weighing 1100 gm., received intravenous injections of 1 cc. of an 18 hour serum-broth culture of this strain P on December 5, 6, 7, 8, 9 and 10, 1938. It died on December 11,

* While this method has resulted in the production of gross endocardial vegetations with all three endocarditis strains employed, it now appears that the disease may sometimes be transmitted by very few injections and there is an indication that a bacterial strain may become more adapted to the rabbit by successive animal passages.

6 days after the first inoculation. At autopsy there was some reddish material adherent to the mitral valve, which was considered as a possible vegetation. Microscopic study of sections through this revealed a laminated clot adherent to the ventricular surface of the leaflet and to the mural endocardium at its base. The deeper part of this deposit contained many leukocytes and on top of this was a layer of red clot. No bacterial colonies were recognized in it. An early lesion of endocarditis was considered possible but not proved. We have not included such animals among our positive results. Rabbits 143, 148, 345 and 472 of this group died in the period December 16 to 27, or 11 to 22 days after inoculations were begun, and at autopsy their hearts appeared negative.

The other 4 rabbits, 469, 304, 302 and 465, died in the period December 23 to January 5, that is, 18 to 31 days after inoculations were begun, and all of these showed convincing gross vegetations on the cardiac valves. These animals were used for testing various therapeutic agents. The protocol of one of them will suffice for our present purpose. Rabbit 469, weighing 1390 gm., was injected intravenously with a serum-broth culture of strain P, 1 cc. daily December 5 to 10 and December 12 to 15, and 1.5 cc. daily December 16, 17 and 19, and 2 cc. daily December 20, 21 and 22, 1938. On December 23 the animal was given 1 cc. of culture intravenously and then by stomach tube 10 grains of sulcamfamide dissolved in 5 cc. of water. Death occurred 15 minutes later. At autopsy the posterior aortic cusp presented a remarkable picture, being thickened to form a mass 5 by 4 by 3 mm., with a perforation of the leaflet at the base of this mass. The right cusp also presented a marginal thickening 1 mm. in diameter. Otherwise the heart appeared negative. In the cortex of the left kidney were numerous hemorrhagic spots and on the surface of the spleen a single yellow elevation 1 by 2 mm. in area. Microscopic sections of the aortic vegetations revealed abundant colonies of streptococci in the fibrin and adjacent necrotic tissue, and within the viable fibroblastic tissue of the valve was a microscopic abscess with a large bacterial colony at its center. This viable tissue was richly infiltrated with leukocytes.

This same strain P was used to inoculate 12 rabbits of a second series, beginning on December 28, with daily intravenous injec-

tions of 4 cc. Some of these were used for experimental therapy. Up to Feb. 12, 1939, 8 of them had died and all of these had recognizable gross vegetations on the endocardium. These rabbits are listed in Table I and photographs of the hearts of Rabbits 303, 307 and 313 are shown in Plate 105. The protocol of one of them will serve to indicate the program of inoculation. Rabbit 316, weighing 1800 gm., received intravenous injections of serum-broth culture of strain P, 4 cc. daily on December 28, 29, 30 and 31 (1938) and January 1, 3, 4, 5, 6, 7, 9, 10 and 11 (1939), a total amount of 52 cc. in 13 injections. The animal died about 1 hour after the last injection. Immediate autopsy revealed 100 cc. of clear watery fluid in the peritoneal cavity, an enlarged spleen, 50 by 14 by 8 mm., a congested liver with lesions of coccidiosis, and about 50 cc. of clear fluid in each pleural cavity. Both lungs were marked by numerous subpleural hemorrhagic spots. The heart was enlarged and both auricles and the right ventricle were enormously dilated. Large vegetations, attaining a thickness of 5 mm., were found obstructing the mitral orifice. Microscopic sections confirmed the gross observations and revealed abundant colonies of streptococci in the mitral vegetations.

A third series of 12 rabbits was started on January 17. All of these were inoculated with cultures of the same strain P. Several of them were used for therapeutic tests. Nine of them had died by March 5, 1939, and the lesions noted at autopsy are indicated in Table I. Six of the 9 had gross endocardial vegetations. The hearts of Rabbits 318, 326, 329, 325 and 354 are shown in Plate 105. The protocol of 1 animal will serve to illustrate the group. Rabbit 325, weighing 2070 gm., received intravenous injections of serum-broth culture of strain P, 4 cc. daily (except Sunday) from January 17 to February 25 inclusive, a total of 116 cc. of the culture. Blood cultures taken on January 23, February 13, 20 and 23 were positive; those taken on January 31 and February 6 remained negative. The weight had fallen to 1870 gm. on February 27. On this day a dose of 250 mg. of sulfapyridine in 5 cc. of 5 per cent gum acacia was given by stomach tube. This dose was repeated twice on February 28 and once on March 1. Death occurred March 1 at 2.30 P.M. At autopsy there were a few small coccidial lesions in the liver. The heart was dilated and there were large mitral vegetations, one of these measuring 4 by 4 by 6 mm.

TABLE I

Data on Experimental Rabbits Inoculated with Endocarditis Streptococci

Rabbit No.	Strain	Initial inoculation	Death	Significant lesions
12	M	Jan. 19, 1937	Mar. 4, 1937	Mitral vegetation
11	S	Jan. 19, 1937	Mar. 12, 1937	Negative
19	M	Jan. 30, 1937	Apr. 12, 1937	Negative
466	W	Feb. 8, 1937	Feb. 11, 1937	Negative
467	G	Feb. 8, 1937	Feb. 15, 1937	Negative
458	P	Dec. 5, 1938	Dec. 11, 1938	Uncertain
143	P	Dec. 5, 1938	Dec. 16, 1938	Negative
148	P	Dec. 5, 1938	Dec. 19, 1938	Negative (?)
345	P	Dec. 5, 1938	Dec. 20, 1938	Negative (?)
469	P	Dec. 5, 1938	Dec. 23, 1938	Aortic vegetation, spleen
472	P	Dec. 5, 1938	Dec. 27, 1938	Negative
304	P	Dec. 5, 1938	Dec. 30, 1938	Mitral vegetations
302	P	Dec. 5, 1938	Dec. 30, 1938	Tricuspid vegetations, kidney
465	P	Dec. 5, 1938	Jan. 5, 1939	Mitral vegetation
316	P	Dec. 28, 1938	Jan. 11, 1939	Mitral, mural vegetations, kidney, lung
314	P	Dec. 28, 1938	Jan. 15, 1939	Tricuspid vegetations
315	P	Dec. 28, 1938	Jan. 16, 1939	Mitral vegetation
303	P	Dec. 28, 1938	Jan. 25, 1939	Mitral vegetation
317	P	Dec. 28, 1938	Jan. 28, 1939	Mitral vegetation
322	P	Jan. 17, 1939	Jan. 30, 1939	Negative
338	F	Jan. 27, 1939	Jan. 31, 1939	Negative
313	P	Dec. 28, 1938	Feb. 1, 1939	Tricuspid, aortic, mitral vegetations
341	P	Jan. 27, 1939	Feb. 1, 1939	Negative
328	P	Jan. 17, 1939	Feb. 2, 1939	Negative
336	F	Jan. 27, 1939	Feb. 3, 1939	Negative
307	P	Dec. 28, 1938	Feb. 4, 1939	Tricuspid, mitral vegetations
342	H	Jan. 27, 1939	Feb. 6, 1939	Negative
332	H	Jan. 27, 1939	Feb. 9, 1939	Negative
309	P	Dec. 28, 1938	Feb. 12, 1939	Mitral vegetation, lung
361	F	Feb. 13, 1939	Feb. 20, 1939	Ante mortem thrombus in auricle
331	P	Jan. 17, 1939	Feb. 20, 1939	Mitral and tricuspid vegetations
320	P	Jan. 17, 1939	Feb. 21, 1939	Ante mortem thrombi in auricles
362	F	Feb. 13, 1939	Feb. 21, 1939	Negative
354	P	Jan. 17, 1939	Feb. 23, 1939	Mitral and tricuspid vegetations
368	P	Feb. 13, 1939	Feb. 24, 1939	Negative
326	P	Jan. 17, 1939	Feb. 24, 1939	Mitral vegetations
318	P	Jan. 17, 1939	Feb. 27, 1939	Mitral vegetations, lungs
325	P	Jan. 17, 1939	Mar. 1, 1939	Mitral vegetations, lungs
380	P	Feb. 13, 1939	Mar. 2, 1939	Negative
378	P	Feb. 13, 1939	Mar. 3, 1939	Negative
329	P	Jan. 17, 1939	Mar. 5, 1939	Aortic and mitral vegetations
372	P	Feb. 13, 1939	Mar. 6, 1939	Negative
343	P	Jan. 27, 1939	Mar. 7, 1939	Mitral vegetations, kidney, lungs
365	F	Feb. 13, 1939	Mar. 7, 1939	Negative
376	F	Feb. 13, 1939	Mar. 15, 1939	Mitral vegetations, lungs, spleen

TABLE I (*Continued*)

Rabbit No.	Strain	Initial inoculation	Death	Significant lesions
310	P	Dec. 28, 1938	Mar. 15, 1939	Mitral lesion
333	H	Jan. 27, 1939	Mar. 16, 1939	Mitral vegetations, lungs
335	H	Jan. 27, 1939	Mar. 18, 1939	Mitral vegetations, kidney
337	F	Jan. 27, 1939	Apr. 5, 1939	Mitral vegetation
373	P	Dec. 28, 1938	Apr. 10, 1939	Decision reserved
399	P	Jan. 17, 1939	Apr. 10, 1939	Decision reserved
468	P	Dec. 5, 1938	Apr. 10, 1939	Decision reserved
306	P	Dec. 28, 1938	Apr. 10, 1939	Decision reserved
311	P	Dec. 28, 1938	Apr. 10, 1939	Decision reserved
319	P	Jan. 17, 1939	Apr. 11, 1939	Decision reserved
323	P	Jan. 17, 1939	Apr. 11, 1939	Decision reserved
381	F	Jan. 27, 1939	Apr. 19, 1939	Mitral vegetations, lungs, spleen, liver

Microscopic sections revealed numerous colonies of streptococci in the vegetations.

Twelve rabbits of a fourth series were inoculated on January 27. Four of these animals received a culture of strain H derived from the blood of a proved case of human endocarditis lenta. All of these died; Rabbit 332 on February 9 after receiving daily intravenous injections from January 27 to February 9 inclusive. The heart of this animal appeared negative at autopsy. The other 3 animals survived for longer periods and all 3 at autopsy showed large endocardial vegetations. Rabbit 334 was the last to die and its protocol may serve to illustrate the entire group. Rabbit 334, weighing 2150 gm., received intravenous injections of a serum-broth culture of strain H, 2 cc. daily on January 27, 28, 30 and 31, and February 1, 2, 3 and 4; 4 cc. daily from February 7 to 23 inclusive; and 6 cc. daily from February 24 to April 15 inclusive. Blood cultures taken at weekly intervals remained persistently negative until April 10. On this date and again on April 13 a blood culture was taken which gave a positive growth. The animal died during the night of April 15 and at autopsy the following morning the spleen was enlarged and the aortic orifice was occupied by massive vegetations.

Four of the rabbits of this fourth series were inoculated with cultures of strain F, likewise from the blood of a patient with proved endocarditis lenta. All are dead. Rabbit 338 died on January 31 after 4 intravenous injections only of 2 cc. each on January 27, 28, 30 and 31. The heart appeared negative. Rab-

bit 336 died on February 3 after 6 injections only. This heart contained a laminated clot attached to the tricuspid valve but microscopic study failed to reveal bacterial colonies. The other 2 rabbits (337 and 381) survived for longer periods and at autopsy showed gross mitral vegetations. Rabbit 381, weighing 2020 gm., received intravenous injections of serum-broth culture of strain F, 2 cc. daily from January 27 to February 18 inclusive; and 4 cc. daily from February 20 to April 17 inclusive. A blood culture taken on January 31 was positive but subsequent weekly blood cultures remained negative until that of April 3, which gave a positive growth. The blood culture taken on April 10 remained sterile but another taken on April 13 became positive. The animal died at 11:40 A.M. April 19, and large vegetations were found in the mitral orifice.

Four of the rabbits of this fourth series were inoculated with serum-broth cultures of strain P. Rabbit 341 died on February 1 after 4 intravenous injections only of 2 cc. each. There was a firm laminated clot in the right ventricle but microscopic examination failed to reveal bacterial colonies. Rabbit 342 died on February 6 after having received 7 intravenous injections of 2 cc. each. There was a small bit of fibrin attached to the mitral valve but bacterial colonies could not be recognized. Rabbit 343 died on March 7 after 31 intravenous injections amounting to a total of 108 cc. of the culture. Blood cultures taken on February 27 and on March 6 were positive. At autopsy the spleen was much enlarged and large vegetations were present in the mitral orifice (Plate 105). Rabbit 340 behaved in a remarkable way. It received 56 injections of the culture, amounting to a total of 208 cc. from February 27 to April 10. Of the weekly blood cultures, those taken on February 6, 27, March 1, 6 and 13 had given a positive growth, but all the others remained sterile. The animal was sacrificed on April 14 and the heart appeared entirely negative in the gross examination.

Discussion

The table lists 57 animals, of which 27 showed recognizable inflammatory lesions of the endocardium. Nearly all of these lesions were quite large and unmistakable on gross inspection. In fact, there is only one of these 27 accepted as positive in which it was really necessary to await microscopic examination. The

photographs illustrate the characteristics of these lesions, which closely resemble those of actively progressive, malignant endocarditis of man, as seen postmortem. There are 23 animals listed as negative without any searching microscopic study of the heart. It is not improbable that significant positive lesions might be found by thorough microscopic study but this has seemed relatively unimportant at the moment. In 7 animals decision was reserved. All of these were animals subjected to prolonged observation during life in accordance with special therapeutic programs and we wish to make more complete studies of the specimens before discussing them further.

It is already evident that endocarditis lenta, a specific infectious disease of man, can be transmitted to rabbits in a large percentage of experiments by the repeated intravenous injection of large doses of the bacterial culture and that the disease thus produced in the experimental animal exhibits to a large extent the characteristic features of the human disease. It seems, however, that the rabbit possesses a relatively high natural resistance to the infection and we believe, along with Rosenow and Lloyd-Jones, that there is sometimes a real tendency for the lesions in the rabbit to heal unless their development and extension is favored by continued repeated inoculations of the culture or by other depressing influences such as coccidiosis. This apparent balance between the forces of infection and resistance would seem to suggest the rabbit as a valuable experimental animal in which to study the phases of extension and of healing of the lesions and especially for study of the possible influence of various therapeutic measures upon such processes. We are attempting observations of this sort. Meanwhile it is hoped that fellow students in this field may find something helpful in the present report.

Summary and Conclusion

1. By repeated intravenous injection of large amounts of pure cultures in serum-broth it has been possible to transmit endocarditis lenta of man to the rabbit.

2. The endocardial lesions in the rabbits have usually been quite large and easily recognizable by gross inspection.

3. Microscopically these lesions resemble those of the human disease. Large colonies of the streptococci are easily found.

4. In the rabbit there is sometimes an evident tendency for these lesions to heal, which suggests that this animal may be of value in testing therapeutic measures against the disease.

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DESCRIPTION OF PLATES

PLATE 105

FIG. 1. Heart of Rabbit 303, cut open and showing large mitral vegetations. A small piece removed from this specimen was used to prepare the section for Figs. 15 and 16.

FIG. 2. Heart of Rabbit 325 with mitral vegetations.

FIG. 3. Heart of Rabbit 329 with small mitral and extensive aortic vegetations.

FIG. 4. Heart of Rabbit 313 with tricuspid, mitral and aortic vegetations.

FIG. 5. Kidney of Rabbit 313 with a recent gross infarct.

FIG. 6. Heart of Rabbit 381 with large mitral and smaller aortic vegetations. A block was taken through the aortic valve before the photograph was made.

FIG. 7. Heart of Rabbit 307 with large tricuspid and smaller mitral vegetations.

FIG. 8. Heart of Rabbit 326 with mitral vegetations.

FIG. 9. Heart of Rabbit 343 with mitral vegetations.

FIG. 10. Heart of Rabbit 331 with mitral vegetations and small vegetations near the attachment of the tendinous cord to the tricuspid.

FIG. 11. Heart of Rabbit 376 with massive mitral vegetations.

FIG. 12. Heart of Rabbit 318 with mitral vegetations. A bit of black silk, inserted to hold the specimen, is seen near the vegetation at the right border of the picture.

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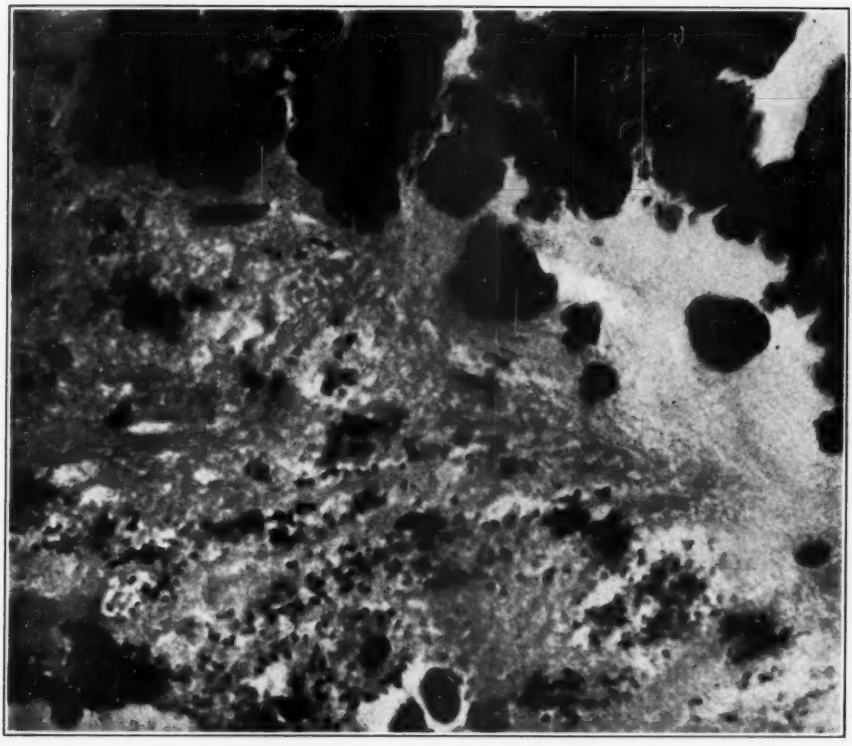
Fig. 13. Heart of Rabbit 337 with mitral vegetations.

Fig. 14. Heart of Rabbit 354 with mitral and tricuspid vegetations. The latter cannot be clearly seen in the photograph.

Fig. 15. Microphotograph of a section through the mitral vegetation of Rabbit 303 (Fig. 1) stained by the modified Gram method of Brown and Brenn. A portion of the auricular wall is seen above and a portion of the ventricular muscle below. The deformed and thickened mitral flap occupies the center of the figure. It presents a confused intermingling of various elements including large bacterial colonies in necrotic granulation tissue and fibrin, with crumbling at the free margin. Here there are a few erythrocytes included in the fibrin and a red clot clinging to the free surface.

Fig. 16. Microphotograph of a small portion of the same section at high magnification. In this region, near the free margin of the vegetation, is a meshwork of cell-free fibrin containing massive bacterial colonies and, toward the free surface, scattered bacteria and a few well preserved erythrocytes.



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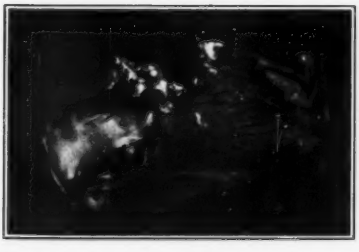

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STUDY OF THE HISTOLOGICAL CHANGES AND TRANSPLANTATION OF TISSUE SURROUNDING METHYLCHOLANTHRENE PELLETS DURING THE LATENT PERIOD OF TUMOR DEVELOPMENT IN FEMALE C_3H MICE *

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When the susceptible tissues of an animal are treated with a carcinogenic hydrocarbon there is a latent period between the first application of the agent and the development of a tumor. The time of tumor induction varies, depending on the agent, the solvent, the tissue treated and the species of test animal employed, and even the genetic constitution of animals of the same species. During this latent period certain of the cells which are exposed to the action of the carcinogenic agent undergo changes antecedent to the development of a frankly malignant process. Since the neoplasms are transplantable in nearly 100 per cent of other mice of the same strain as the animal in which they were originally induced, an experiment was planned by which a comparison could be made between the results of histological examination and transplantation of the tissue around a carcinogenic agent at weekly intervals during the latent period of tumor development when the local cells are assuming the characteristics of autonomous growth.

MATERIALS AND METHODS

The mice employed in this study were of the inbred C_3H strain obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. They were maintained on a diet of dog chow exclusively, except for the first several days after being received in this laboratory during which time their diet was supplemented with bread and milk. Water was allowed freely at all times. 203 mice were set aside for the experiment. Of these, 81 females aged 5 to 7 months were inoculated subcutaneously into the anterior abdominal wall with methylcholanthrene pellets implanted by means of a trocar and cannula. The pellets contained 5 per

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cent of methylcholanthrene and 95 per cent of cholesterol. The technic followed in making the pellets has been described by Shear.[1] Pellets of cholesterol alone prepared by this method and injected subcutaneously into C_3H mice do not evoke tumors. In several thousand mice of the C_3H strain observed under experimental and control conditions in this laboratory during the past several years there has been no instance of the occurrence of a spontaneous sarcoma in the anterior abdominal wall in the experience of the author. The methylcholanthrene-cholesterol pellets were cylindrical in shape and each weighed between 12 mg. and 20 mg. and contained therefore between 0.6 mg. and 1 mg. of methylcholanthrene. At weekly intervals during a period of 98 days 3 pellet animals and in one instance each, 4 and 1 pellet animals a week, 41 in all, were killed and autopsied. The pellet was found in the abdominal wall in 34 of these animals and in the peritoneal cavity in 7. The remaining 40 of the 81 original pellet mice were not used for transplantation purposes because of extensive ulceration of the abdomen, disappearance of the pellet or death. The routine practice was to excise the anterior abdominal wall and spread it out on a cork with the hair side down. In those instances in which the pellet was found in the peritoneal cavity the lesion was widely excised and pinned down on a cork. By means of a pair of iris forceps and fine pointed scissors part of the tissue surrounding the pellet was excised and used for transplantation into other C_3H mice. The remainder of the tissue with the pellet in place was dropped into fixative and later trimmed in thin pieces which were blocked separately. In obtaining the tissue adjacent to or in contact with the pellet care was exercised to avoid chipping the pellet or including fragments of it in the transplant. However, since the complete absence of methylcholanthrene from the transplant was not proved in any instance in the present experiment, certain of the results are open to the possible interpretation that in their new environment the transplanted cells may have been exposed to the continued effects of concealed hydrocarbon in the transplant. Similarly, certain of the tumors which arose in the recipient mice may possibly have been evoked by particles of the carcinogenic agent accidentally carried over in the graft.

Histologically the character and intensity of the reaction of

the tissues surrounding a methylcholanthrene-cholesterol pellet vary from area to area. Therefore, in order to secure more uniform results it was the practice to select for transplantation samples of any areas of thickening or of otherwise unusual appearance. The excised tissue from each pellet mouse was grafted into the anterior abdominal wall of 3 other strain C_3H mice by the trocar method. A total of 122 mice, of which 94 were males, was used for transplantation. The mice that received the transplants were examined by palpation each week and any notable changes recorded, including the date on which the successful transplants first showed active growth. The pellet tissue in all cases, and all the transplants which developed into tumors, as well as many specimens of the abdominal wall of mice in which the transplant receded or failed to grow progressively, were examined grossly and microscopically. Most of the tissue preserved for histological study was fixed in Zenker's fluid; occasionally Bouin's or Tellyesniczky's fluid, or dilute formaldehyde solution was used. The tissue was blocked in paraffin, cut at 7 μ, and stained with hematoxylin and eosin. Other stains also used on occasion were phosphotungstic acid hematoxylin, eosin-methylene blue, Foot's silver stain for reticulum, and Mallory's aniline blue connective tissue stain. Since methylcholanthrene and cholesterol are dissolved in the reagents employed in the paraffin technic, the stained histological sections did not contain the pellet but instead there was a space which corresponded in size and shape to the position of the pellet in the living animal.

Certain terms are used in the following pages which require explanation. The mice into which pellets were injected are referred to as pellet mice or the pellet group; the term positive is applied to those which yielded grafts which grew on transplantation, whereas the opposite is indicated by the designation negative. The mice into which the transplants were inoculated are designated the recipient mice or recipient group, and the terms positive or negative are employed respectively to denote those which did or did not develop a progressively growing tumor at the site of inoculation. In the stained histological sections the space occupied by the pellet in the living animal is referred to as the pellet space.

Case Histories and Protocols

At 28 Days: Transplants of the pellet tissue of Mouse 24 were made into 3 mice (Nos. 70, 74 and 76). Microscopic examination of the pellet tissue revealed that the superficial half of the space was lined by a layer of squamous epithelium which was continuous with the skin surface overlying the pellet. This portion of the skin was thickened and the nearby hair follicles showed atrophy and hyperplasia. A few dilated mammary gland acini near the pellet were lined by flattened squamous epithelium. There were focal and diffuse chronic inflammatory changes with hemorrhage, pigment and numerous basophilic leukocytes. Some small veins were thrombosed. Inside the pellet space were keratin, macrophages and leukocytes in various stages of degeneration and necrosis. Of the 3 mice receiving transplants, 1 (No. 74) was of interest. This animal showed a slight swelling at the site of inoculation a month later which persisted for a few weeks but failed to progress. At autopsy 105 days later there was a small squamous lined cyst on the inner aspect of the abdominal wall at the site of inoculation.

At 42 Days: Pieces of the pellet tissue of Mouse 37 were excised and transplanted into 3 mice (Nos. 77, 81 and 92). Histological examination of the abdominal wall containing the pellet revealed an inflammatory process surrounding the pellet space, which occupied a position in the subcutaneous tissue immediately below the panniculus carnosus. Superiorly the space was narrowly separated from the base of the hair bulbs. The deeper portion of the pellet space was in relation to the fat and the abdominal musculature. The pellet space was immediately surrounded by several layers of collagenous fibers containing large vacuolated phagocytic leukocytes and a few elastic fibers. One of the sections included the area immediately adjacent to that which was excised for transplantation. This area was composed of a chronic inflammatory reaction with granulation tissue, polymorphonuclear and mononuclear leukocytes, foreign body giant cells, lymphocytes and fibroblasts, and a few mammary gland acini, some of which showed squamous metaplasia. In this area were small acicular spaces surrounded by degenerated polymorphonuclears. Among the fibroblasts were a few spindle shaped cells which were atypical

in appearance with heavy nuclear walls and large deeply stained aggregations of chromatin material in the nuclei which contained relatively large nucleoli. The thin superficial muscle bundle within the superficial fascia showed atrophy, necrosis and hyperplasia of some of its fibers near the pellet. One small area of the deeper musculature in contact with the pellet was necrotic and showed an inflammatory reaction. There was a decrease in hair follicles and elastic fibers in the skin overlying the pellet. Of the 3 mice inoculated with the pellet tissue 2 (Nos. 77 and 92) lived for 161 days and showed no evidence of growth of the transplant by the end of that time. The remaining mouse (No. 81) showed beginning growth of the transplant at 98 days and progressive enlargement for 49 days, when the animal was killed and showed a spindle cell sarcoma.

At 49 Days: Bits of tissue from around the pellet of Mouse 1 were transplanted into 3 mice (Nos. 115, 118 and 119). Microscopically the pellet space, containing masses of necrotic cells and fibrin, lay beneath the panniculus carnosus and above the deep musculature (Fig. 1). It was separated from the latter by fibrofatty tissue containing mammary gland ducts and acini, a few of which showed squamous metaplasia. Individual fibers overlying the pellet space showed necrosis and hyperplasia; occasional muscle fibers contained exceedingly large hyperchromatic nuclei occupying a central position in the cell. Around the pellet space was a collagenous membrane lined internally partly by vacuolated phagocytes and partly by a layer of squamous epithelium. External to this were focal and diffuse chronic inflammatory changes. One area at the lower pole of the pellet space contained numerous atypical hyperchromatic spindle cells. Of the 3 mice that received the transplant, 2 were negative and 1 (No. 119) was positive. One (No. 115) of the negative mice showed beginning enlargement of the transplant at 63 days, this condition being maintained for 2 weeks, after which the lesion regressed. The positive mouse (No. 119) showed beginning growth of the transplant at 70 days. Growth continued steadily for 42 days when the animal was killed and showed a spindle cell sarcoma (Fig. 2).

At 77 Days: Bits of tissue surrounding the pellet of Mouse 133 were excised and transplanted into 3 mice (Nos. 179, 184 and

185). Microscopic examination of the lesion showed the pellet space completely surrounded by a connective tissue membrane lined by a thin layer of squamous epithelium over two-thirds of its inner circumference. Outside the pellet space and extending laterally in the subcutaneous tissue was a chronic inflammatory reaction with abundant granulation tissue, hemorrhage, fibrin and necrosis. This area contained isolated hyperchromatic spindle shaped cells with thick nuclear walls within which there was an abundance of coarse chromatin granules. These cells resembled closely those of the tumors which developed from the transplants. Atypical spindle cells showing pronounced mitotic activity were also observed developing in relation to the layer of deep fascia adjacent to the musculature of the body wall separated by some little distance from the capsule of the pellet (Fig. 3). Several nerve fibers were thickened and hyperplastic. A few fibers in the deep musculature and several in the panniculus carnosus showed degenerative and hyperplastic changes. The hair follicles near the pellet space showed diminution and atrophy and a few were enlarged and filled with keratin. The overlying skin showed hyperkeratosis adjacent to the ulcer but no marked thickening. Of the 3 mice which received the transplant 1 (No. 184) died 91 days later without showing any signs of growth at the site of inoculation, while the other 2 developed tumors. One of these mice (No. 185) showed growth of the transplant on the 56th day, with progressive enlargement for 3 weeks when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 179) was found dead 91 days after inoculation and showed a spindle cell sarcoma.

At 82 Days: Bits of tissue from a nodule adjacent to the pellet of Mouse 148 were transplanted into 3 male C_3H mice (Nos. 189, 190 and 192). Microscopic examination of skin in the region of the pellet revealed two nodular lesions near each other in the subcutaneous tissue. One of these was an adenosquamous cell carcinoma and the other a spindle cell sarcoma. Of the 3 mice which received transplants 2 were positive and 1 was negative. Of the 2 positive mice, 1 (No. 190) showed evidence of beginning growth of the transplant at 1 week and continued growth for 49 days when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 189) showed beginning growth

of the transplant at the 9th week and was killed 2 weeks later and showed a spindle cell sarcoma.

Bits of tissue adjacent to the pellet of Mouse 143 were transplanted into 3 mice (Nos. 194, 195 and 196). Histological examination of the pellet tissue revealed a space surrounded by the fibrofatty tissue of the omentum which showed a chronic inflammatory reaction. Within the space were groups of degenerated and necrotic cells, chiefly polymorphonuclear leukocytes and vacuolated mononuclear cells. The inflammatory reaction was circumscribed and contained an abundance of granulation tissue, leukocytes, a few of which were eosinophilic, and mononuclear, small round and spindle cells which appeared to be fibroblasts. Of the 3 mice receiving transplants 2 were positive and 1 was negative. One positive mouse (No. 195) showed beginning growth of the transplant at 21 days. The lesion grew progressively for 84 days when the mouse was killed and showed a spindle cell sarcoma (Fig. 4). The remaining mouse (No .196) also showed beginning growth of the transplant at 21 days. The lesion enlarged for 126 days when the animal was killed and showed an atypical giant cell sarcoma.

At 91 Days: Bits of this tissue from about the pellet of Mouse 130 were implanted into 3 mice (Nos. 198, 201 and 204). Microscopic examination of the pellet lesion showed most of the section to be composed of areolar tissue and a few small fragments of inflammatory tissue. The invading cells were of various types, chiefly mononuclears and small round cells. There were many spindle cells with large, deeply stained, thick walled nuclei containing numerous large chromatin particles. There was considerable collagen in the lesion. A few focal areas of inflammatory reaction occurred about acicular spaces. Of the 3 mice which received transplants, 2 were positive and 1 was negative. One of the positive mice (No. 198) showed beginning growth of the transplant 2 weeks following injection and progressive enlargement for 49 days when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 201) showed beginning growth of the transplant at 42 days, with rapid enlargement of the lesion for 21 days when the animal was killed and showed a spindle cell sarcoma.

At 98 Days: Bits of tissue from about a small nodule adjacent

to the pellet of Mouse 134 were implanted into 3 mice (Nos. 206, 207 and 208). Microscopically the small peritoneal nodule was composed of atypical spindle cells, chiefly small round cells and polymorphonuclear leukocytes diffusely distributed throughout the tissue. The nuclei of the spindle shaped cells were oval to rod shaped, a few were round and others elongated with pointed ends. Mitotic figures were extremely numerous, averaging over three per high power field. The nuclei had thickened walls and generally contained a large nucleolus and coarse chromatin particles. The cytoplasm was fibrillar and not very abundant. The cells were usually arranged in more or less distinct parallel bundles and sheets running in various directions, in a few places forming distinct interlacing bundles. The blood vessels, which were not numerous, resembled capillaries or were slit-like and thin walled. There were a few areas of necrosis. Several voluntary muscle fibers with a chain of multiple nuclei in the middle and striations in the fibers were observed in the cellular portion of the nodule. At the periphery the nodule was not well demarcated but instead the peripheral cells infiltrated the surrounding fat. The lesion was suggestive of an early malignant process but a positive diagnosis was not justified on histological grounds. Of the 3 mice which received transplants of the pellet tissue 2 were positive and 1 (No. 208) was negative. Of the 2 positive mice 1 (No. 206) showed beginning growth of the transplant at 14 days and rapid progressive enlargement for 14 days when the animal was killed and showed a spindle cell sarcoma. The remaining mouse (No. 207) likewise developed a spindle cell sarcoma at the site of the tissue inoculation at the 14th day which grew rapidly for 2 weeks when the mouse was killed.

Results

The results of the experiment are presented in Table I and Table II. All the positive transplants were sarcomas. The case histories and protocols of the mice of the pellet and recipient groups which gave positive results are presented under a separate heading.

A 42 day pellet mouse yielded the earliest fragment of tissue which grew progressively on transplantation and developed into a tumor. The next positive transplant was obtained at 49 days.

From 49 to 77 days there was a lapse of 4 weeks during which none of the transplants gave positive results. Of the 3 pellet mice sacrificed each week from 77 to 91 days, 1 in two instances and 2 in one instance yielded tumor transplants in 2 of the 3 recipient mice used in the respective experiments. Tumors grew in 2 of the 3 recipient mice transplanted with tissue from a pellet mouse at the 98th day. In no instance did all 3 mice receiving transplants excised from around a single pellet develop tumors at the point of

TABLE I

Results of Transplantation of Tissue Surrounding 5 Per Cent Methylcholanthrene-Cholesterol Pellets in Strain C_3H Mice

Number of mice implanted with pellets	Period in days between implantation of pellet and transplantation of tissue	Number of recipient mice	Maximum number of days mice with transplants survived	Number of mice developing tumor from transplant
3	7	9	196	0
3	14	9	189	0
3	21	9	182	0
3	28	9	175	0
3	35	9	168	0
3	42	9	161	1
4	49	12	154	1
3	56	9	105	0
3	63	9	224	0
3	70	9	154	0
3	77	9	112	2
3	82	9	196	4
3	91	8	133	2
1	98	3	126	2

inoculation. Positive results were obtained in 3 of 7 mice in which the pellet was found in the peritoneal cavity at autopsy.

Seventeen mice which received transplants but which were regarded as negative since they failed to develop a tumor, subsequently showed a variable degree of enlargement at the site of inoculation. These cases were distributed as follows: 3 during the first half of the experiment and 14 in the latter half. There was a latent period of from 2 to 11 weeks, averaging 4 weeks, in 8 of these cases during which the transplant remained stationary before enlarging. The duration of enlargement was difficult to estimate in 6 cases, and in the remainder (11 cases) growth continued from 2 to 7 weeks, averaging 4 weeks. In some instances regression was practically complete by the end of the experiment,

the transplant being completely absorbed so that no visible change was found grossly or microscopically at the site of inoculation. In 4 cases a small nodule persisted to the end of the experiment. One of these showed enlargement during the first 3 weeks only and at postmortem examination a nodule 5 by 8 mm. was present in the abdominal wall. On microscopic examination the skin and peritoneum were uninvolved and the lesion consisted of a cyst-like inflammatory area in the subcutaneous tissue, without any evidence of malignancy. Another case showed growth for 1

TABLE II

Data on Pellet Tissue which Yielded Successful Transplants

Number of pellet mouse	Period in days between implantation of pellet and transplantation of tissue	Number of positive recipient mouse	Days after tissue transplantation that progressive growth was first noted	Duration of progressive growth of transplant	Average diameter in mm. of transplant at autopsy
37	42	81	98	49	20
1	49	119	70	42	22
133	77	185	56	21	16
		179	91	?	5
148	82	190	7	49	16
		189	63	14	10
143	82	195	21	84	12
		196	21	126	10
130	91	198	14	49	8
		201	42	21	10
134	98	206	14	14	9
		207	14	14	10

week. At postmortem examination 21 weeks later there was a small yellow elevated nodule 3 by 4 mm. in the subcutaneous tissue. Microscopic examination revealed a small area of fibrosis with no suggestion of malignancy. In the 3rd case the transplant became palpable at 9 weeks, showed enlargement for 2 weeks and then decreased in size but did not completely disappear. At postmortem examination there was a small gray nodule 2 mm. in diameter in the subcutaneous tissue of the abdominal wall. Microscopically this consisted of an encapsulated area of fibrofatty tissue infiltrated with mononuclear leukocytes and lymphocytes but with no evidence of malignancy. In the remaining case a small squamous cell lined cyst was found at the site of inoculation (see Mouse 24 at 28 days).

Six female mice in this experiment developed adenocarcinoma

of the mammary gland. These neoplasms corresponded grossly and microscopically to the mammary carcinomas which develop spontaneously in a high percentage of the females of this strain. Other spontaneous lesions which occurred in some of the mice during the experiment were pneumonia, chronic nephritis, *Klossiella muris* infection of the kidney and prostatic abscess.

Discussion

This experiment was designed to determine at what point, during the malignant transformation of tissue in contact with a 5 per cent methylcholanthrene-cholesterol pellet implanted subcutaneously into strain C_3H mice, the cells in the pellet tissue would produce a progressively growing malignant tumor when transplanted subcutaneously into other mice of the same genetic constitution. These results were correlated with the histological changes occurring in the pellet tissue. Employing the same technic, Andervont [2] obtained tumors between 8 and 22 weeks (average 14.5 weeks) in 22 of 25 female strain C_3H mice injected with 5 per cent methylcholanthrene-cholesterol pellets in the subcutaneous tissue of the right axillary region. In the present study the pellets were allowed to remain in the mice from 1 to 98 days which is approximately the average latent period for tumor development under the conditions of the experiment.

Histological and cytological studies of the premalignant changes in the subcutaneous tissue around a carcinogenic hydrocarbon have shown that atypical cells arise in different locations in relation to the active agent and that these resemble the cells of the fully developed neoplasm (Wolbach,[3] Hval,[4] Rondoni,[5] Lewis,[6, 7] and Shear [8]). Shear [1] obtained tumors in recipient mice inoculated with implants excised from the reactive tissue on the 47th day after injection of carcinogenic hydrocarbons and thereafter. In the present experiment little or no correlation could be established between the histological characteristics of the pellet tissue and its successful transplantability in some instances, whereas in others the incidence of atypical cells about the pellet was so high that it was not surprising that transplantation was successful. In control studies in which plain cholesterol pellets were injected into the subcutaneous tissue the lesions showed only a chronic foreign body reaction. In the histological sections of the pellet tissue from

the positive pellet mice in the present experiment atypical cells with characteristics resembling malignant cells were absent in 1 case (Mouse 143 at 82 days), occasionally found in 1 case (Mouse 37 at 42 days) and numerous in 3 cases (Mice Nos. 1, 133 and 130 at 49, 77 and 91 days respectively). In 1 case (Mouse 134 at 98 days) the numerical increase in these cells was so great and their structural arrangement so significant that the lesion in the pellet tissue was suggestive of malignancy. The remaining animal (Mouse 148 at 82 days) showed two small neoplasms near the pellet, one an adenosquamous cell carcinoma, the other a spindle cell sarcoma. Both transplants which developed from grafts obtained from this last mouse were sarcomas. The fact that atypical cells in pellet tissue were observed in profusion in the histological sections from many mice sacrificed before the 42nd day and from several others from 42 to 98 days whose transplants failed to yield tumors in the recipient mice, suggests that other factors in addition to malignant appearing cells about the hydrocarbon are needed for the development of a tumor upon their transplantation into a new host. However, the results of the transplantation studies indicate that malignant changes may be induced in cells exposed to the carcinogenic action of methylcholanthrene before the necessary criteria for the histological recognition of malignancy become fully established.

Positive transplants of pellet tissue were obtained from pellet mice sacrificed at 42 and 49 days and, following a lapse of 4 weeks, at 77, 82, 91 and 98 days. From a study of tumor development in the positive recipient mice it is evident that the property of autonomous growth which cells assume during the evolution of malignancy may be delayed or inhibited when they are separated too early from the carcinogenic agent which initiates this property and are transferred into the new environment afforded by another host. Thus, in several instances in the positive groups the longer the tissue transplant remained in contact with the carcinogenic agent in the pellet mouse, the more apt it was to be successful on transplantation, the less time was required for it to show the first sign of progressive growth, and the more rapid its growth in the host animal. This is illustrated by the pellet mice sacrificed at 42 and 49 days which yielded transplants requiring 98 and 70 days respectively to show beginning growth in the host; whereas in the

case of pellet mice sacrificed at 91 and 98 days the tissue transplants came up at 14 and 14 days respectively. Secondly, the respective transplants from each of the 2 positive pellet mice at 42 and 49 days developed into tumors in only 1 of 3 recipient mice, as compared with 2 of 3 recipient mice receiving tissue inoculations from each of the 5 positive pellet mice from 77 to 98 days. Finally certain individual transplants in the later group (77 to 98 days) grew relatively much more rapidly after their inception than did those in the earlier group (42 to 49 days, Table II). These findings therefore substantiate the view that the adaptation of the transplant to the new host, its growth rate, and its latent period decrease inversely with the length of time the pellet tissue is allowed to remain in contact with the carcinogenic agent.

In most instances when two transplants grew from the same pellet tissue the histological appearance of the two tumors was similar. An exception to this, however, occurred at 82 days. In this experiment Mouse 143 was found to have the pellet in the peritoneal cavity and microscopic examination of the pellet tissue revealed a chronic inflammatory process with relatively few malignant cells. Two of the 3 mice receiving transplants of this pellet tissue developed tumors at the point of implantation. Histological examination of the tumors revealed that 1 mouse had a spindle cell sarcoma composed of relatively uniform spindle shaped cells, while the growth in the other was composed of atypical spindle cells together with numerous tumor giant cells. Since the methylcholanthrene pellet is of sufficient size so that it invariably comes in contact with different tissues along its course, such as blood vessels, nerves, skeletal muscle, connective tissue, skin, peritoneum, and so on, hyperplastic and neoplastic changes may therefore occur in the cells composing various structures.

It is believed that the tumors arising following inoculation of tissue surrounding the pellet were the result of inoculation of malignant cells and not the result of inoculation of small amounts of the carcinogenic agent. In Table II it is seen that in one instance a period of 98 days elapsed between the inoculation of the test material and a palpable growth. Inasmuch as the concentration of methylcholanthrene contained in the inoculated material must have been very minute, if present at all, a period of 98 days is an extremely short latent period for the induction of tumors by

minute quantities of methylcholanthrene. Of 122 animals of the recipient group 110 failed to develop a progressively growing tumor at the site of inoculation of the transplant. If sufficient methylcholanthrene was diffused throughout the tissues from the pellet of Mouse 37 to produce a tumor one would expect to obtain more than 12 tumors when tissues from the area surrounding other pellets were placed in the subcutaneous tissues of 122 other animals.

Seventeen mice in the negative recipient groups exhibited transitory enlargement of the graft at some time or other during the period of observation following transplantation. The causes for this cannot be assigned in each individual case but there would seem to be several factors which might have a bearing on it. The results might have been more homogeneous had the separate transplantation fragments surrounding each pellet been finely ground and pooled before injection. Moreover, in certain cases the inflammatory reaction invariably present in pellet tissue might account for the failure of its cells to become established in the new host. Considering these and perhaps other factors to play a part in this phenomenon it is conceivable that as many as 17 out of 110 mice in the negative recipient group might exhibit temporary local enlargement at the site of inoculation due to inflammatory changes which might also inhibit the establishment of the transplant in the new host. However, when the distribution of these cases over the range of the experiment is considered it is seen that 14 of them occurred among 46 negative animals in the latter half of the experiment from 56 to 98 days inclusive, as against only 3 out of 64 negative animals in the first 49 days. This fact arouses speculation as to whether this transitory enlargement of the transplants may not have been due in part to the temporary growth of cells on the border line of malignancy in an abortive attempt to form a tumor. This hypothesis is supported by the fact that the 14 cases of transitory enlargement occurred at a time when 10 transplants developed into tumors in recipient mice in contrast to the 3 transitory cases of enlargement among a group of only 2 positive transplant mice in the first half of the experiment. Furthermore, if enlargement in this group were due entirely to inflammatory change, it would be expected that this reaction would occur within a relatively short time after transplantation.

This was true in 8 cases in which enlargement followed immediately on transplantation and in 1 case the record is incomplete. However, in the others there was a latent period of 2 weeks in 3 cases, of 3 weeks in 2 cases and of 6, 9 and 11 weeks respectively in 3 cases each. Therefore, in at least 8 of these cases consideration has been given the possibility that the graft contained cells which exhibited temporary signs of proliferation following a definite latent period but failed for one reason or another to develop into fully formed tumors.

Summary and Conclusions

1. Five per cent methylcholanthrene-cholesterol pellets were placed subcutaneously in C_3H mice. The purpose of the experiment was to compare the results of histological examination and of transplantation of the tissue around a carcinogenic agent at weekly intervals during the latent period of tumor development.

2. Tumor-yielding transplants were obtained from pellet mice sacrificed at 42 and 49 days and following a lapse of 4 weeks at 77, 82, 91 and 98 days.

3. In the histological sections of the pellet tissue which did give rise to progressively growing tumors when transplanted, atypical cells with characteristics resembling malignant cells were present in varying number in the different mice.

4. The results suggest that other factors in addition to the presence of malignant appearing cells about the hydrocarbon are needed for the development of a tumor upon transplantation of pellet tissue into a new host. The results of the transplantation studies indicate that malignant changes may have been induced in cells exposed to the carcinogenic action of methylcholanthrene before the necessary criteria for the histological recognition of malignancy became fully established.

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DESCRIPTION OF PLATE

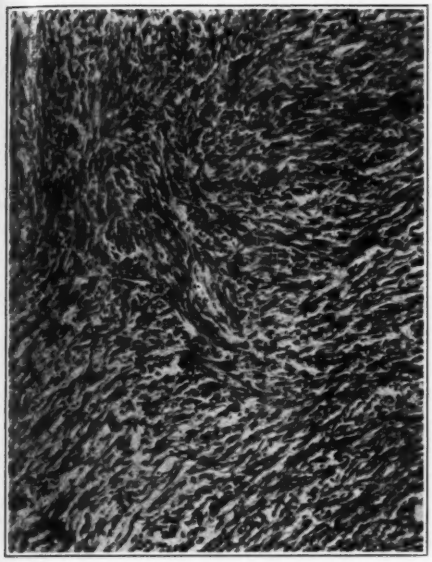
PLATE 107

FIG. 1. Anterior abdominal wall of Mouse 1 inoculated with a methylcholanthrene-cholesterol pellet 49 days previously. The pellet space is in the subcutaneous tissue with the skin above and the deep musculature below. The pellet space is partially surrounded by thickened tissue and on the left border is lined by a thin layer of squamous epithelium which appears partially detached in the illustration. There is a loss of hair follicles over the pellet. × 17.

FIG. 2. Spindle cell sarcoma from Mouse 119. This tumor arose in the abdominal wall at the site of inoculation of a fragment of tissue excised from the pellet capsule of Mouse 1 at 49 days. Histological section of the pellet tissue from Mouse 1 is illustrated in Fig. 1. × 140.

FIG. 3. Mouse 133 injected 77 days previously with a methylcholanthrene-cholesterol pellet. The area is from the deep fascia some little distance removed from the capsule of the pellet. There is an infiltration of a few lymphocytes, mononuclear and polymorphonuclear leukocytes, and proliferation of numerous atypical hyperchromatic spindle cells. Three mitotic figures are present near the center of the illustration. × 350.

FIG. 4. Section of the spindle cell sarcoma of recipient Mouse 195. This tumor grew from implants obtained from the pellet tissue of Mouse 143 which was injected 82 days previously with a methylcholanthrene-cholesterol pellet. The pellet in Mouse 143 was found in the peritoneal cavity rolled up in a mass of omentum. × 140.



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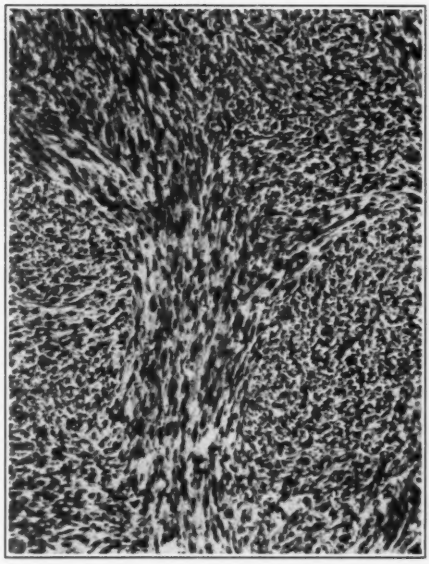

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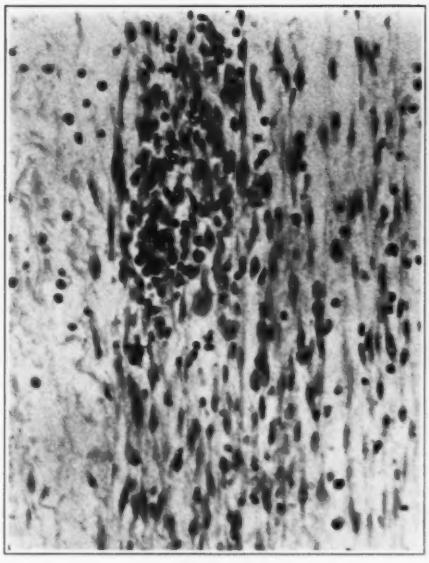

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Stewart

Tissue Surrounding Methylcholanthrene Pellets

STUDIES ON EXPERIMENTAL RICKETS IN RATS*

III. The Behavior and Fate of the Cartilage Remnants in the Rachitic Metaphysis

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Introduction

In describing the bones of rachitic infants practically all writers mention a region just below the proliferative zone of the epiphyseal cartilage which is composed of osteoid tissue and marrow interspersed with islands and projecting masses of cartilage. This is the region which is generally known as the rachitic metaphysis (Text-Fig. 1).

There is no general agreement as to the behavior and fate of these cartilage remnants. It is variously stated in textbooks of pathology that they become changed into osteoid, or that they become encased in osteoid, or that their margins change into osteoid. In none of the papers on experimental rickets has this question received much attention. In a paper on rickets in rats Pappenheimer [1] declines to discuss the "difficult question of the direct metaplasia of cartilage into osteoid tissue." Lobeck [2] considers that in experimental rickets in rats these remnants become incorporated into bone trabeculae, the cartilage cells thereby becoming surrounded by bone and subsequently being transformed into bone cells. Park [3] states that these cartilage masses become changed into "pseudo-osteoid, . . . which greatly resembles the osteoid tissue produced by the osteoblasts."

In 1935 [4] we reported briefly on the question but in a later, more extensive paper [5] omitted reference to the matter. We consider the behavior of these masses of considerable interest, not merely as part of the pathological process, but also as having a bearing on the fundamental relations between bone and cartilage.

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This paper presents more mature observations, clearing up some questions and pointing the issue on certain others.

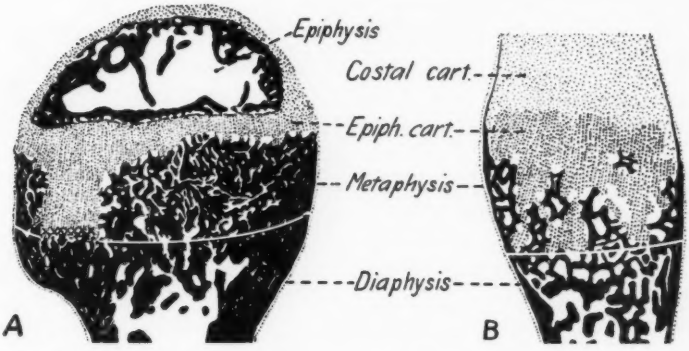
Material and Methods

This paper is based on continued study of the material used in earlier papers on rickets. Use was made of 125 albino rats; 31 with active rickets, 68 in various healing stages, and 26 healthy animals. Rickets was produced by the Steenbock-Black rachitogenic diet with high calcium, low phosphorus and deficient vitamin D. Healing was induced either by irradiated ergosterol (Viosterol) or cod liver oil. In some of the rats healing occurred spontaneously. The rachitogenic feeding was begun at 4 weeks and the administration of the curative agents at 8 or 9 weeks. Both longitudinal and transverse sections were cut at 10μ after decalcification in Müller's fluid. Hematoxylin and eosin stains were used. Our main study was on the head of the tibia, with frequent comparisons with other bones. The behavior of the cartilage remnants in the rachitic bones followed essentially the same course whether in active rickets or in early healing stages. The later healing stages furnished important information about the relation between cartilage and osteoid in the metaphysis of rachitic bones.

The Rachitic Metaphysis. Its Genesis and Nature

It is now generally recognized that the rachitic metaphysis is produced by partial removal of the thickened epiphyseal cartilage by the agency of highly vascular marrow from the diaphysis. The process is excellently described by Park.[3] The cartilage cells and the uncalcified cartilage matrix are destroyed with about equal rapidity. The cells degenerate, in the main by pyknosis (Fig. 11), although a few undergo fragmentation and others fading. The invading narrow tongues bore irregular holes in the cartilage, leaving anastomosing cartilage trabeculae of varying thickness, but all large enough to include entire cells as well as matrix. Before long it is seen that there is considerable osteoid associated with the cartilage remnants while at the same time much of the cartilage seems to have disappeared (Fig. 1). The question under consideration is, *does the cartilage change into osteoid?* The location and general extent of the rachitic metaphysis are shown in Text-Fig. 1.

It should be remembered that the cartilage composing these remnants is not of the active growing type, but rather of the kind that makes up the greater part of the rachitic epiphyseal cartilage. The cells are large and are generally considered to be well on the



TEXT-FIG. 1. Drawing showing general structure of bones with well advanced rickets. The open spaces represent marrow. In both bones the greatly thickened epiphyseal cartilage has been partly replaced by the characteristic structures of the rachitic metaphysis. The trabeculae of the metaphysis (shown in black) are composed at the one extreme of persisting portions of the epiphyseal cartilage, and at the other of well developed osteoid, as illustrated in Figs. 1 to 12. Within the irregular trabeculae there runs, with no great distortion, the parallel trabeculae of the epiphyseal cartilage. The region is wholly uncalcified or very nearly so. The trabeculae of the diaphysis and of the epiphysis are composed of persisting prerachitic bone covered with osteoid.

A = Head of the tibia from a white rat, 12 weeks old, with severe rickets. From a tracing over a photograph. × 10. XXXX indicates the position of cartilage rejuvenation described on page 731.

B = Costochondral junction in well developed rickets. Adaptation of a figure in a current textbook of pathology, directly comparable to the head of the tibia in Text-Fig. 1 A, except that removal of the cartilage has not been carried so far and the metaphysis is consequently less extensive.

way to disintegration. The cytoplasm is highly vacuolated and faintly basophilic; the nucleus is large, about spherical, and has widely separated chromatin granules. The lacunae have become correspondingly enlarged and the intervening matrix in many places is reduced to thin lamellae (Figs. 1, 2, 3, 9 and 11). In severe rickets this cartilage is without calcification. In both the

main cartilage masses and the smaller remnants the cells have retained their arrangement in longitudinal columns of definitely limited length, except where the weakened cartilage has suffered crushing or distortion by mechanical pressure.

These cartilage trabeculae vary in thickness, the thinner ones including but a single layer of cell columns, while the thicker trabeculae and the intersections have four or five. The following description does not deal with the behavior of the much larger cartilage masses, some of which are always present in the rachitic bones (Text-Fig. 1).

The Behavior and Fate of the Cartilage Remnants During Active Rickets

It will be noticed in Figure 1, a typical area from the rachitic metaphysis, that in the upper part of the picture the newer cartilage remnants have the same typically cartilaginous structure as the main cartilage mass from which they spring, whereas those lower down, the progressively modified portions, come to look more and more like osteoid tissue. We will now describe the extent to which cartilage gives place to osteoid and the manner in which this occurs. In this general change we have observed the operation of three general processes — infiltration, envelopment and internal reorganization.

1. The Infiltration of Cartilage with Osteoid

It is evident that it is only the extreme tips of the marrow masses that have the power of rapid destruction of cartilage. At a short distance below the front the destructive action is limited to the opening of some of the superficial lacunae on the surfaces of the persisting cartilage trabeculae and the destruction of the contained cells, but the formation of osteoid is going on rapidly. These opened lacunae are promptly invaded by osteoblasts, usually one in each lacuna, which have in this region developed in large numbers in the marrow from the cells surrounding the blood vessels. Within each such lacuna there promptly builds in from the periphery toward the center a layer of osteoid matrix, surrounding the enclosed osteoblast (Fig. 3), the deposition continuing until there remains only a small lacuna occupied by the

new osteoid cell. During the filling of the cartilage lacunae with the osteoid matrix the cytoplasmic processes of the osteoblasts, in the usual manner, become surrounded from apex to base by the new matrix, thus forming the canaliculi extending outward from the reduced lacunae (Fig. 10).

The new matrix thus formed within the opened cartilage lacunae usually stains but faintly with eosin, although in some filled lacunae the new matrix shows a deeply staining, granular zone. Thus the superficial portions of the cartilage remnants become infiltrated with osteoid. In the new tissue formed in this manner the pattern of the persisting cartilage matrix shows clearly within the new osteoid formation (Fig. 3).

This tissue might well be called osteoid filled cartilage. It is quite comparable in its mode of formation to the intrachondrial bone described by Bast [6] in the human otic capsule. We are not sure, however, whether any distinction should be made between endochondrial and intrachondrial ossification, inasmuch as both types involve the laying of bone matrix upon a base of cartilage matrix, the only difference being the extent of the cartilage matrix involved. In both types the cartilage matrix persists visibly within the new formation. The concept is nevertheless a useful one and Bast has made a useful contribution in pointing out the exact nature of the process occurring in the otic capsule.

2. The Envelopment of Cartilage Remnants by Osteoid

When the superficial lacunae have been filled by osteoid, as described above, the osteoblasts continue to build more osteoid on that already deposited in the lacunae. In other areas this enveloping layer of osteoid is laid directly on the surface of cartilage trabeculae whose superficial lacunae have not been opened. In either condition the osteoid thus formed may build up to considerable thickness, thus reducing the bore of the marrow canals. This process is most active in a zone of the metaphysis a short distance from the cartilage removal front.

The tissue thus formed is a typical well developed osteoid. It differs from that formed by infiltration of cartilage in that it contains none of the cartilage matrix, that its cells are placed rather at random instead of with a strong tendency to linear arrange-

ment, and that its matrix stains very deeply with eosin, so that it shows as dark areas in photographs (Figs. 1, 2, 4 and 8).

3. *The Internal Reorganization of Cartilage*

The foregoing two types of tissue are direct products of the marrow, inasmuch as the cells are derived from the marrow and the matrix is formed under the direct influence of these cells. The type now to be described has a quite different origin, being derived from the cartilage.

We have observed that when the cartilage trabeculae are not more than one or two cell columns in thickness all lacunae are usually promptly opened and filled with osteoid as already described, but when the masses are three or more cells in thickness there are some internal cells that remain unchanged for a longer time (Figs. 1, 2 and 3). These deeper cells are shut off from immediate contact with the marrow by the superficial lacunae which have been filled with osteoid and by the enveloping layer of osteoid. But if the cartilage masses are not too large these internal cells are not wholly beyond the influence of the marrow, as indicated by the behavior now to be described.

After considerable study of many sections we have become convinced that these hypertrophied cartilage cells in unopened lacunae undergo a form of rejuvenation together with a transformation in type. Many of them undergo mitotic division, take on the appearance of osteocytes, and there forms about them within the unopened lacunae a matrix that has the appearance of osteoid matrix such as forms in the superficial opened lacunae. Thus there is produced a tissue which is largely indistinguishable from that formed by infiltration under the influence of osteoblasts from the marrow (Fig. 8). Several lines of evidence support this view:

(*a*) *Many Lacunae Do Not Become Opened:* After microscopic study of sections, including a series of complete serials through the metaphysis of rachitic bones, it has become definitely apparent that the lacunae under discussion do not become opened. Cross sections of bones have been particularly convincing, inasmuch as such sections enable one to trace the trabeculae of cartilage and the columns of cartilage cells, and thus to determine with certainty which have been opened and which have not (Fig. 2).

(*b*) *Many of the Cartilage Cells Divide by Mitosis:* Two to four cells are seen in many of the unopened lacunae in these remnants, although only one is present in each lacuna in the general mass of cartilage from which the remnants have come (Figs. 2, 4, 5 and 7). In explanation of this condition mitotic figures of normal appearance are frequently seen in cells which are clearly of the cartilage type. We have observed both first mitoses, making two cells, and second mitoses, making four cells within one lacuna (Fig. 6). The number of dividing cells varies from rat to rat.

(*c*) *The Cartilage Cells Undergo Transformation into Cells Resembling Osteoid Cells:* This transformation is indicated by the presence of wide gradations of cells in these unopened lacunae, from typical cartilage cells at the one extreme, to cells very much like osteoid cells at the other. These intermediate type cells occur mainly in an intermediate position in the metaphysis. Close to the parent cartilage are found cells only of the cartilage type, while toward the end of the shaft they are mainly of the osteoid type (Fig. 1). This distribution of cell types, when observed repeatedly, leads one to the conclusion that the various intermediate forms are indeed actual intermediate stages leading toward a fairly uniform final type.

The transformation involves change in the staining reaction of the cytoplasm from basophil to acidophil, condensation of the cytoplasm, the development of cytoplasmic processes, and the condensation of the nucleus (Figs. 2, 4, 5 and 7). In general these cells, when fully transformed, are smaller than osteoid cells from the marrow and their cytoplasm is scant and likely to be vacuolated. Their cytoplasmic processes are more slender than those of true osteoid cells. These cytoplasmic processes do not develop by growth but rather are strands of cytoplasm which have retained contact with the capsule of the lacuna while the general mass of cytoplasm underwent condensation. Both kinds of cells are variable and frequently they cannot be distinguished unless it can be seen whether the lacuna has or has not been opened. The transformation begins before the first mitotic division and is continued after the divisions have been completed. Some of the cells, while still of large size, show numerous eosinophilic droplets in the cytoplasm, similar to those described by Pappenheimer [1] in

the ribs of rachitic rats. We do not know their significance but apparently only a small proportion of the cells ever have them (Fig. 5). There are other variations in the details of the transformation but the foregoing account gives a general picture of the process.

(*d*) *Matrix Forms About These Cells:* While the cartilage cells are undergoing the changes just described the cartilage matrix surrounding the lacunae in which they lie remains undistorted. The lacunae do, however, grow smaller, the change in size being due to the deposition of new matrix within the cartilage lacunae. It appears first as a thin layer in each lacuna, adherent to the capsule, while the cells are yet large and more like cartilage cells than osteocytes in appearance. As the cells grow smaller the matrix increases proportionally (Figs. 4, 5, 6, 7 and 8). The growing matrix progressively surrounds the cytoplasmic processes from apex to base, thus forming canaliculi extending out from the lacunae in which the new osteoid cells lie. When the cartilage cells have divided, producing more than one cell in a cartilage lacuna, some of the new matrix usually forms also between the cells so that each cell thus comes to occupy a separate lacuna. The new matrix in some lacunae stains but faintly with eosin (Fig. 8), while in others it has a zone of granules which stain strongly with eosin (Fig. 7). This deposit is not a thickening of the capsule surrounding the lacuna. The two are of different nature, as indicated by their microscopic appearance, their staining reaction and their behavior during healing of rickets.

The product of these changes is a tissue which is neither cartilage nor osteoid, but with greater resemblance to the latter. Its general staining reaction is pale (Figs. 1 and 8). The cartilage matrix pattern persists. The cells are usually small and look, after their rejuvenation, as if they were once more on the road to degeneration. It must be recognized, although we have not demonstrated it microscopically, that the cytoplasmic processes and the canaliculi in which they lie terminate for each cell at the inner surface of the capsule of the cartilage lacuna, each cell thus failing to communicate by canaliculi with adjacent cells as do the cells of true osteoid. In this respect the tissue resembles cartilage whose cells lack the communicating channels of bone and osteoid.

This tissue resembles the infiltration type of osteoid in that both have the cartilage matrix within the osteoid matrix, and in the usually pale stain of the new matrix. But we are usually not able to distinguish between the two unless the section be cut in a plane to show whether the lacuna has been opened. The two as a group are, however, easily distinguishable from the enveloping osteoid (Fig. 8).

It should be remarked that neither infiltration osteoid nor enveloping osteoid is formed on the large cartilage masses except under special conditions. The surface of such masses usually remains in direct contact with marrow, as if cartilage removal were more or less actively in progress. It was only the smaller masses, not more than about five cells in thickness, which were under the influence of the marrow in such a way as to become covered or filled with osteoid tissue.

In the preceding paragraphs we have described a rejuvenation of cartilage cells, coupled with a transformation into a form and functional activity like that of osteoblasts. Lest the idea of rejuvenation of cartilage cells in rachitic bones may seem unreasonable, or supported by inadequate evidence, we wish again to call attention to a different type of rejuvenation of cartilage cells (Dodds and Cameron [7]) which we have observed frequently and clearly in 43 of the same rats in which we have observed the changes just described. This rejuvenation was recognized also and clearly illustrated by Harris.[8] This process takes place in the main mass of the thickened cartilage close to the end of the diaphysis, the area being marked XXXX in Text-Fig. 1 A. In these areas the hypertrophied cartilage cells become restored to the appearance of cells in healthy growing cartilage: the nucleus becomes smaller and denser, the cytoplasm grows more compact and more sharply contoured, but no cytoplasmic processes develop. The cells undergo mitotic division, giving rise to typical groups of two or four, as in regular cartilage growth, but quite unlike the special flattened cells in the growth zone of the epiphyseal cartilage. The matrix also becomes adjusted to the expanding groups of cells and the characteristic capsules form about the lacunae. The total picture is quite different from the filling process observed in the remnants in the metaphysis. The fact of rejuvenation of hypertrophied cartilage cells is, however, clearly

demonstrated. Comparison of Figures 8 and 9 shows clearly how different are the results of the two kinds of rejuvenation.

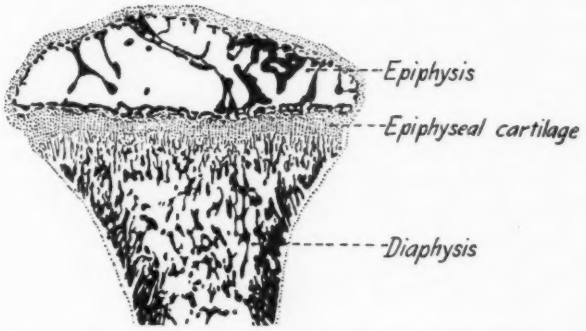
Cartilage Remnants During the Healing Process

Further light is thrown on the fate of the cartilage remnants by study of healing stages, while calcification is taking place and while the orderly bone trabeculae of the normal spongiosa are taking form in the regions formerly occupied by the chondro-osteoid trabeculae of the rachitic metaphysis.

When calcification is resumed three kinds of calcified matrix are seen in the region of the metaphysis. (1) Calcified cartilage matrix, which stains very deeply and uniformly with hematoxylin and which shows as black in photographs (Fig. 12, C). It follows the pattern of the earlier uncalcified cartilage matrix within the osteoid filler except that the capsules immediately surrounding the lacunae remain uncalcified. The general result is that the longitudinal matrix walls between the columns of cells become calcified, while the transverse partitions between adjacent lacunae within each column remain uncalcified. This is similar to the distribution of calcification in the epiphyseal cartilage of normal bones. (2) Calcified osteoid filler of cartilage lacunae. This portion of the matrix, when calcified, shows clearly about each osteoid cell as a zone of deeply stained granules, separated by a paler zone, the uncalcified capsule, from the dark area of the calcified cartilage matrix (Fig. 12, F). This includes both the infiltration osteoid and that formed by internal reorganization of cartilage tissue. (3) Calcified matrix of enveloping osteoid. This kind of osteoid often occurs in large masses. Calcification appears as clouds of deeply staining granules of varying density (Fig. 12, E).

During the early stages of healing the calcified osteoid filler and the calcified enveloping osteoid are removed, in large part at least, leaving the calcified cartilage matrix as a base for bone deposition. We have made no special study of the fate of the osteoid cells when the matrix is removed. They probably degenerate. Nor have we determined conclusively whether this calcified osteoid is wholly removed or if a small portion persists in the new bone trabeculae. As healing progresses the confused pattern of the chondro-osteoid trabeculae which characterizes the rachitic metaphysis gives place to the rather parallel trabeculae

which characterize the regions in normal bones (Text-Fig. 2). This greater regularity has not been produced by the formation of new trabeculae but rather by the removal of the large amount of osteoid, both enveloping and filling, which during rickets has masked the orientation of the cartilage trabeculae. The cartilage matrix has not been changed into osteoid matrix but much of it has persisted with remarkably little distortion to become the dominant structural element in restoring normal configuration of



TEXT-FIG. 2. Head of the tibia from a white rat 12 weeks old. Complete healing of well developed rickets. From a tracing over a photograph. × 10. The somewhat parallel bone trabeculae of the region just below the epiphyseal cartilage have been produced largely from the earlier rachitic metaphysis, the present orientation being determined by the remnants of cartilage matrix which, during rickets, were enclosed in the irregular osteoid masses. This figure serves in most respects also to represent a normal bone which has never shown the rachitic lesion.

trabeculae in the healing bone. After the delay occasioned by the diseased condition the cartilage matrix has performed its normal function in the formation of the early bone trabeculae. This influence of the cartilage matrix is just as effective in the region of the metaphysis, where for a time the cartilage was submerged in osteoid, as it is in regions where the thick cartilage has never taken part in the formation of the metaphysis, except that the distortion due to pressure is somewhat greater in the region of the metaphysis.

We believe that the foregoing paragraphs in large measure answer the question as to the fate of the cartilage remnants in the metaphysis of rachitic rats, although possibly some of the

cells behave in yet other ways than those described. There is every reason to believe that in the main the same conditions will be found to prevail in human rickets when adequate material has been carefully studied.

Discussion

The foregoing account of the behavior of the cartilage remnants in the rachitic metaphysis portrays, in the main, processes which are quite comparable to those familiar changes in the normal development and growth of bones which result in the deposition of osseous tissue on cartilage remnants. The exceptional feature is the formation of osteoid matrix in unopened lacunae containing rejuvenated cells. We do not know of any description of this process, although several investigators have written rather indefinitely about the metaplasia of cartilage into osteoid tissue.

Our present conclusions are in part different from those expressed in our abstract [4] where it was stated: "We have not observed the deposition of osteoid in unopened cartilage lacunae." At that time we believed that the filling of opened lacunae with osteoid under the influence of osteoblasts from the marrow accounted for the entire mass of cartilage remnants, although soon thereafter our observations brought us to our present conclusions.

Concerning the several conflicting opinions as to the fate of these cartilage masses, it now seems clear that they do become incased in osteoid, that their superficial lacunae become filled with osteoid (not changed into osteoid), and that their internal portions become changed into osteoid only in a very restricted sense. Our observations explain the statement of Lobeck [2] that these remnants become incorporated into bone trabeculae (they become incased in osteoid which later becomes calcified), and that the cartilage cells later are transformed into bone cells (they change into temporary osteoid cells). In all probability these osteoid filled areas are "the transitions which undoubtedly occur between atypical cartilage and osteoid" (Pappenheimer [1]). These rejuvenated and transformed cartilage cells may possibly be the "low-grade bone corpuscles" of Harris [8] in the cartilage islands of rachitic bones, although he gives no adequate description.

Unquestionably this tissue is the "pseudo-osteoid" of Park,[8] although neither he nor any other writer has expressed the same

conception of the genesis and nature of the tissue which we have; *i.e.*, the deposition of new osteoid matrix within the framework of the cartilage matrix. His description leaves no doubt that he has observed the transformation stages of cartilage cells but with different conclusions than ours as to their significance. "We used to think," he writes, "that in rickets actual metaplasia took place, *i.e.*, that cartilage cells, having been reduced to the size and appearance of bone corpuscles, became such. Further studies, however, have convinced us that they actually died." He does not mention mitoses of these cells, nor the other evidences of renewed vitality which we have noted. On the other hand, we concede that their rejuvenation is but transient, inasmuch as they are all seemingly destroyed along with the matrix when healing takes place. We believe that Park's term, "pseudo-osteoid," might very properly be applied to the condition we have described.

In view of the persistent belief that at times cartilage undergoes a direct metaplasia into bone or osteoid, a brief discussion of the points involved in such a change may be in order.

It should be clearly recognized that calcification of the cartilage matrix does not in itself constitute transformation into bone. Such calcification leaves still unchanged the typical structure of the cartilage cells; nor does it cause the matrix to assume the structural nature of bone matrix. Calcification is not the same as ossification, although the two terms are still frequently and wrongly used interchangeably. In normal bone development the matrix of the cartilage becomes calcified in advance of, and as a foundation for, bone deposition, but the cartilage matrix does not become bone although it becomes incased in bone.

When osteoid matrix is formed by osteoblasts in opened lacunae of the cartilage of rachitic bones this cannot be called metaplasia. But it unquestionably is metaplasia when cartilage cells in unopened cartilage lacunae assume the form of osteoid cells, and osteoid matrix is formed about them within the lacunae. The cells have assumed the morphological characteristics of osteocytes, and inasmuch as the matrix formed about them resembles osteoid matrix rather than cartilage, it is clear that they are functioning as osteoblasts. Yet the persistence of the original cartilage matrix within the new osteoid matrix is sufficient to cause the tissue as a whole to fall short of complete metaplasia. So far as we know,

no author has heretofore clearly pointed out the persisting identity of the cartilage matrix within the osteoid of the metaphysis, a point we consider of definite significance. It is thus evident that metaplasia of cartilage involves several elements, each of which must be considered separately.

We may now well consider the four different fates which may befall the cartilage in rachitic bones, with definite relation to regional location and probable external influences: (1) complete and prompt destruction; (2) osteoid infiltration; (3) osteoid rejuvenation; and (4) cartilage rejuvenation. Text-Figure 1 shows the regions now to be considered. In this connection there appears to be justification for the belief of Park [3] that "the cartilage cell cannot be exposed to the influences of blood and remain cartilage."

Cartilage is typically an avascular tissue. Prompt destruction of cartilage cells and matrix takes place at the most advanced portions of the marrow tongues where the raw eroded surface of the cartilage is in immediate contact with the marrow. The marrow at the very front is composed almost wholly of endothelial channels containing blood (Fig. 11). Sometimes, but not often, there are a few primitive connective tissue cells in advance of the vessels. Often there are erythrocytes outside the vessels in the recently opened cartilage lacunae. It would seem indeed that, as assumed by several recent writers, the vascular tissue is the active agent of cartilage destruction. It will be recalled in this connection that in the cartilage removal zone of the normal epiphyseal cartilage the blood channels are in intimate contact with the cartilage, but succeed in destroying only the uncalcified portion, *i.e.*, the cells and the transverse walls within cell columns. The calcified portions apparently are destroyed only by the osteoclasts (Dodds [9, 10]).

Connective tissue cells become abundant between the blood vessels and the cartilage masses at a short distance back from the front of the marrow tongues. Some of these cells soon become osteoblasts on the surface of the cartilage. This is the region of "incomplete exposure to blood" (Park [3]). In such regions cartilage removal ceases except for the opening of the more superficial lacunae and the destruction of their cells. Extensive deposition of osteoid occurs both in the opened lacunae and on the general surface of the cartilage masses. Moreover, it is in such regions that there takes place the transformation of the deeper cartilage

cells, and the deposition of osteoid matrix in unopened lacunae. It appears that the osteoid forming influence of the marrow is not limited to the surfaces in immediate contact with osteoblasts, but also extends to the inner cells of the cartilage remnants, causing them to behave like osteoblasts.

Our observations of the transformation of these cartilage masses exemplify the statement of Park [3] that "the closer to blood vessels, the more the condition resembles osteoid and the further away the more numerous are the cartilage cells which still retain normal features."

After considerable osteoid has been formed on the cartilage remnants and the marrow spaces have become smaller the osteoblasts disappear and osteoid formation ceases. By this time the transformation of the deeper parts of the cartilage masses has also become well advanced.

During active rickets the interior of the large masses of cartilage is clearly beyond the range of influence of the marrow, but it is of interest that in such cartilage, near the cartilage-shaft junction, there often takes place the type of cartilage rejuvenation which produces cartilage. This region is not immediately adjacent to any large masses of marrow but it is not far away from smaller marrow channels in the diaphysis whence there undoubtedly diffuse substances into the adjacent cartilage. One would like to know whether the diffusing substances are different in nature from those reaching the cartilage remnants in the metaphysis, or if they are only less abundant so that cartilage instead of osteoid results from the rejuvenation.

The four types of cartilage reaction just enumerated seem to be determined by the marrow, as if there were a definite gradation or zonation of influence. In part this influence must be through the power of the blood to carry nutrient materials to the tissues and to carry away wastes. But other factors must also be involved such as specific action of various cells in the marrow — the vascular elements, the osteoblasts, the osteoclasts, and probably other unidentified cells. The whole complex of processes is highly involved and embraces the entire scope of the differentiation of the mesenchymal derivatives which participate in bone formation.

In this connection one might ask whether the influence of the marrow is first on the changing cartilage cells and through them

on the matrix, or if both cells and matrix show direct influence from the marrow. Obscure as are these points, they are no more so than are the intimate questions of normal ossification, which in considerable measure still await the attention of the anatomist and the biochemist.

Summary

The cartilage remnants in the rachitic metaphysis of experimental rats were observed to behave as follows:

1. Considerable osteoid is deposited on the surfaces of the cartilage remnants — osteoid envelopment of cartilage.

2. Osteoid is deposited in opened lacunae near the surface of these cartilage remnants by the action of osteoblasts which enter the opened lacunae from the adjacent marrow — osteoid infiltration of cartilage.

3. Cartilage cells farther below the surface of the remnants undergo rejuvenation; many of them divide mitotically and assume the form of osteocytes. While this change is going on, uncalcified osteoid matrix is deposited about them in the unopened lacunae — internal osteoid reorganization of cartilage.

4. In the foregoing internal reorganization process the cartilage cells undergo a metaplasia into osteocyte-like form, but the matrix of the cartilage is not changed. It persists, to become calcified during the healing of rickets and to serve as a base for the deposition of bone in the formation of the trabeculae of the restored bone.

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DESCRIPTION OF PLATES

In all photographs the uncalcified cartilage matrix surrounding the cartilage lacunae shows darker than the uncalcified osteoid matrix which has filled the cartilage lacunae. The enveloping eosinophilic osteoid shows as definitely darker than the osteoid which fills the cartilage lacunae. All photographs are from the head of the tibia. All figures except Figure 12 show active rickets.

1 = Cartilage matrix; 2 = osteoid matrix in opened lacuna, infiltration osteoid; 3 = osteoid matrix in unopened lacuna, transformation osteoid; 4 = space between cell and matrix; 5 = osteoblast or osteoid cell in opened lacuna, infiltration osteoid; 6 = enveloping osteoid; 7 = osteoblast; 8 = hypertrophied cartilage cell; 9 = cartilage cell in transformation to osteoid cell form in unopened lacuna; and 10 = cartilage cell which has been rejuvenated as cartilage cell.

p = Cartilage cell undergoing pyknosis (Fig. 11); e = erythrocytes in advance of nucleated marrow cells at invasion front (Fig. 11); C = calcified cartilage matrix (Fig. 12); F = calcified osteoid filler of cartilage lacunae (Fig. 12); E = calcified enveloping osteoid (Fig. 12); and UE = uncalcified enveloping osteoid (Fig. 12).

PLATE 108

FIG. 1. Edge of cartilage and part of metaphysis of a rachitic rat 8 weeks old. Projecting from the general cartilage mass are the persisting trabeculae of the cartilage, composed of the usual hypertrophied cells occupying lacunae separated usually by only thin walls of cartilage matrix. Farther from the cartilage mass these cartilage trabeculae give place to the different forms of osteoid. The true osteoid (enveloping osteoid) shows darkly in the photograph; osteoid filling the cartilage lacunae is usually lighter

in color. Some marrow spaces are empty because portions of the section have dropped out. × 100.

FIG. 2. Portion of a cartilage trabecula from a rachitic rat 8 weeks old showing early phase of transformation. The cartilage matrix is not calcified although it is deeply stained. On the surface is a layer of enveloping osteoid (6) being built up by the action of the osteoblasts (7). A few of the superficial cartilage lacunae have been opened and osteoid deposited (2). Most of the cartilage lacunae have not been opened (8). Some cartilage cells are still unchanged, others are in various early transformation stages, and some have divided. A little osteoid filler has been formed in a few unopened lacunae (not clearly seen in photograph). × 450.

FIG. 3. Margin of cartilage trabecula from a rachitic rat 8 weeks old. The superficial lacunae have been opened and are being filled with osteoid (2). Osteoblasts from the marrow occupy the opened lacunae and also lie on the general surface of the new osteoid (5, 7). A few unchanged cartilage cells remain within the mass (8). × 950.

FIG. 4. Margin of a cartilage remnant from a rachitic rat 8 weeks old showing osteoblasts on the surface (7) and depositing enveloping osteoid (6). This section shows a series of transformation stages of cartilage cells into osteoid cell form in unopened lacunae (U). Some of the cartilage cells have divided. Osteoid filler is present in varying amounts in these lacunae, even where the cartilage cells have changed but little. Other lacunae have been opened and are now occupied by osteoid filler and by osteoid cells from the marrow (O). × 450.

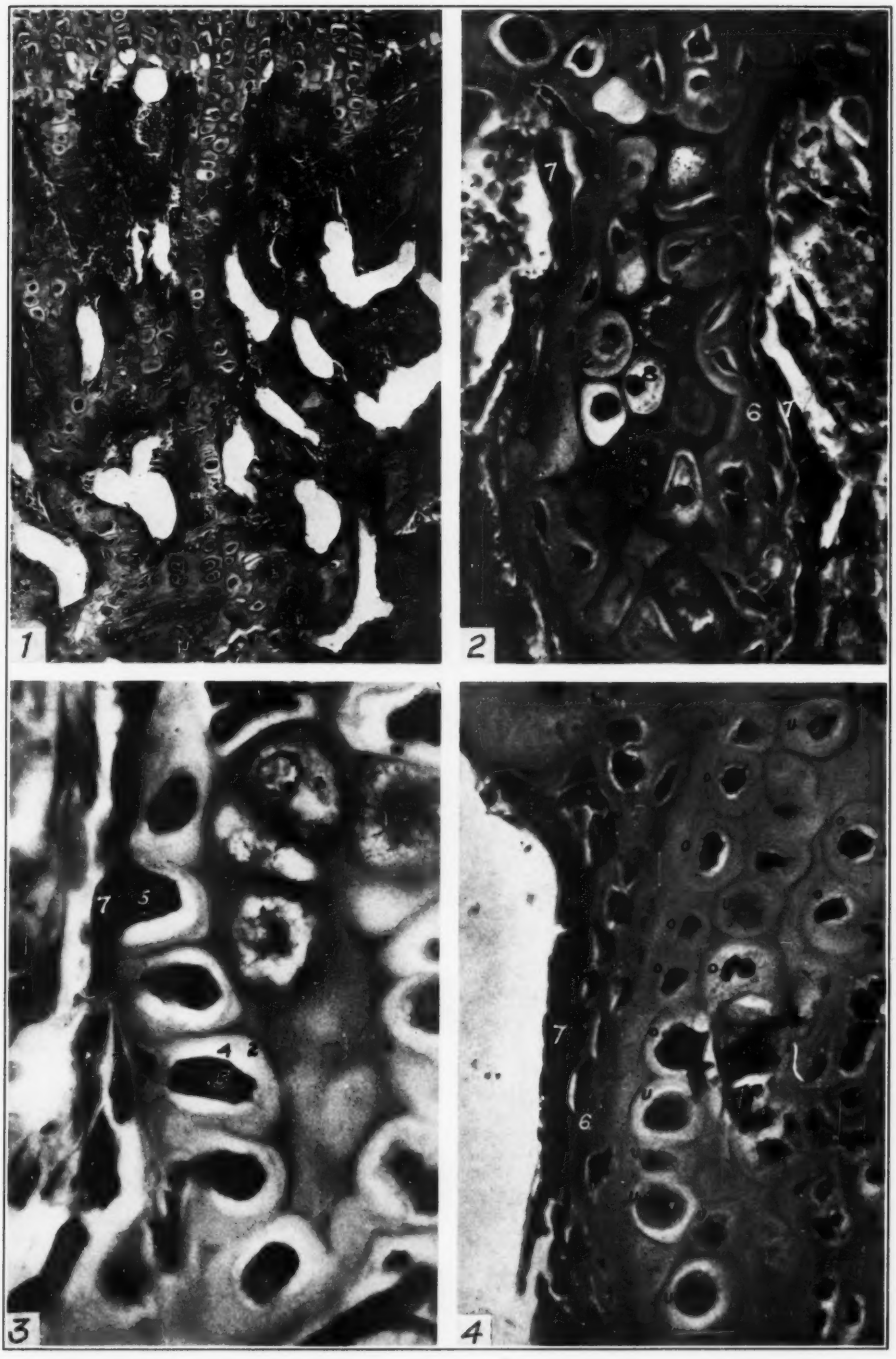
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FIG. 5. Transforming cartilage cells in unopened lacuna from a rachitic rat 8 weeks old. The first mitosis has been completed. These cells show the eosinophilic droplets (dark spots) in the cytoplasm. Some osteoid filler has already been formed about the cells in the lacuna (3). × 950.

FIG. 6. Second mitosis of a cartilage cell in an unopened lacuna from a rachitic rat 8 weeks old. The other cell has completed the second division. A thin layer of osteoid filler is seen in this lacuna (3). × 950.

FIG. 7. Showing transformation of cartilage cells and osteoid filling more advanced than in Figure 4. From a rachitic rat 7 weeks old. Most of the cartilage cells have divided. Considerable osteoid matrix has been formed in the unopened lacunae (3). In some the inner zone of new matrix shows eosinophilic granules (dark color), a condition sometimes seen in the filler of both open and closed cartilage lacunae. One of these lacunae has been opened (2). Enveloping osteoid is also seen in some areas (6). This area is from the lower part of Figure 1. × 450.

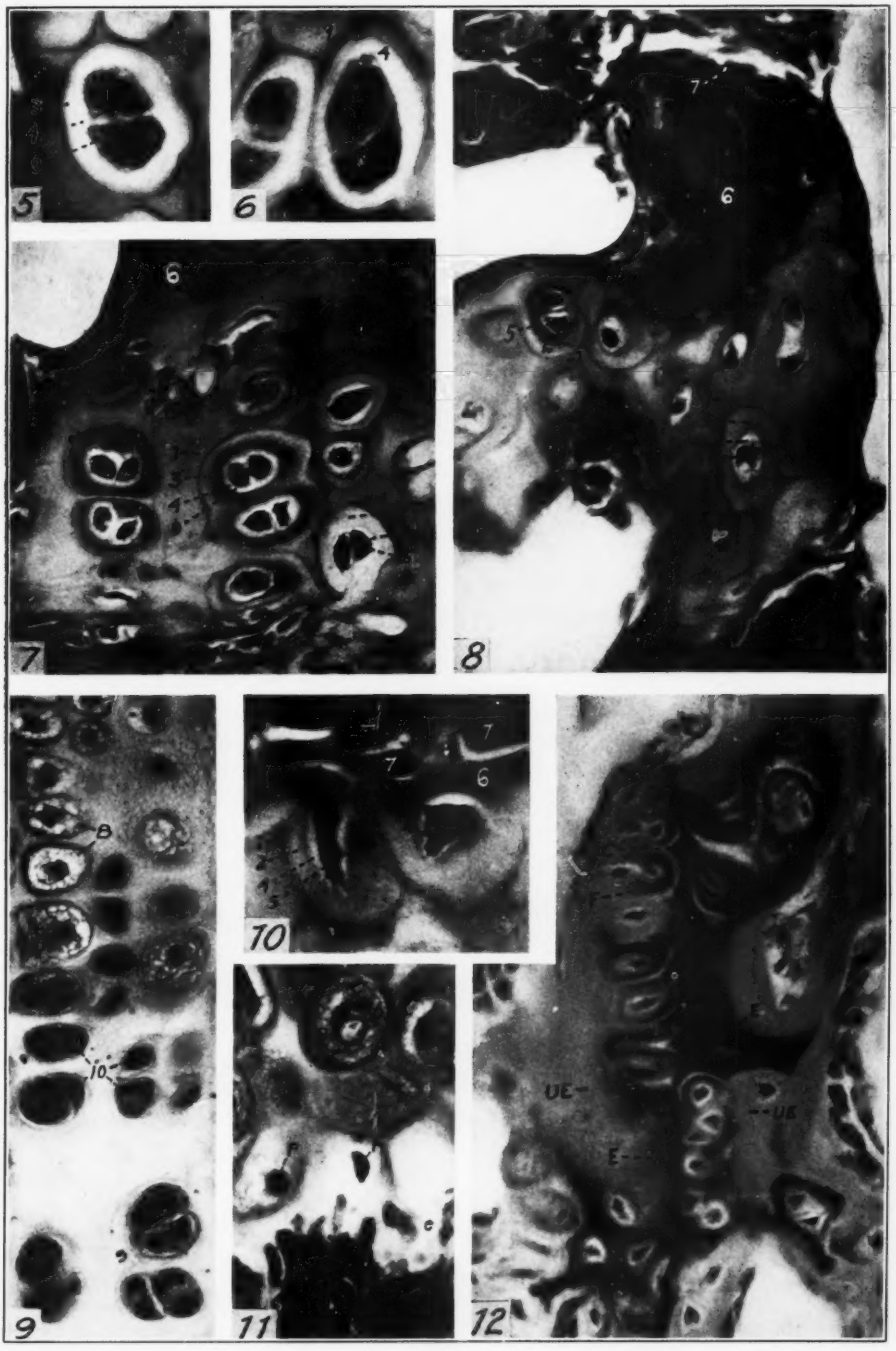
FIG. 8. Portion of a cartilage remnant showing well advanced osteoid filling of an unopened lacunae (light areas) from a rachitic rat 8 weeks old. Shows adjacent enveloping osteoid (6). Osteoblasts (7) are still laying enveloping osteoid in some regions. In the filled area the cartilage matrix pattern (1) shows clearly, as well as the osteoid filler of the lacunae (3). The cells of this area do not appear as vigorous as in the adjacent enveloping osteoid — a common condition. At least one of the lacunae in this field has been filled by infiltration (2). × 450.

FIG. 9. Cartilage rejuvenation in the deeper part of the thick cartilage adjacent to the end of the shaft. From a rachitic rat 10 weeks old. Shows ordinary hypertrophied cartilage cells (8) and others in various stages of rejuvenation (10). Some of the cells have divided once, others twice. The rejuvenated cells appear and act like cartilage cells, producing a tissue which looks like cartilage but not like osteoid. It is very different from the light area in Figure 8. × 450.

FIG. 10. Margin of cartilage remnant from a rachitic rat 8 weeks old. Shows two osteoblasts (5) about which the osteoid matrix is still being deposited in the lacunae (2). These cells show cytoplasmic processes extending into the canaliculi in the newly formed osteoid matrix. Enveloping osteoid (6) is also being deposited by osteoblasts (7) above these cells on the general surface. × 950.

FIG. 11. Edge of cartilage where it is being destroyed by marrow. From a rachitic rat 7 weeks old. Shows the usual hypertrophied cartilage cells (8). Two of these are undergoing pyknosis (p), one in an unopened, the other in an opened lacuna. The marrow shows erythrocytes (e) at the very front followed closely by various nucleated marrow cells. × 450.

FIG. 12. Portion of a trabecula in metaphysis when healing is well under way. From a rachitic rat 9 weeks old. The calcified areas may be differentiated as follows: calcified cartilage matrix, deep and uniform black (C); calcified osteoid filler, dark granular areas surrounding cells (F); and calcified enveloping osteoid, dark granules in varying density (E). Portions are still uncalcified and lightly colored in the photograph (UE). This area suggests Figure 2 and others. × 450.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ESSENTIAL AND PAROXYSMAL HYPERTENSION, CONTRASTED BY CASE REPORTS *

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(From the Letterman General Hospital, San Francisco, California, and the Walter Reed General Hospital, Washington, D. C.)

Articles dealing with essential hypertension are omnipresent in current medical literature. A number of cases of paroxysmal hypertension have been reported and another isolated example would add nothing to our knowledge of this subject. It is my intention to contrast essential and paroxysmal hypertension, hoping thereby to simplify their differential diagnosis.

Essential hypertension of the so-called benign and malignant phase is by reason of its secondary pathology one of the most frequently encountered lethal factors, accounting for approximately 11.4 per cent of all deaths.† Paroxysmal hypertension was the lethal factor in less than 0.1 per cent of the same series of cases. Paroxysmal hypertension is a clear-cut pathological-physiological entity representing the physiological response to neoplastic hyperplasia of normally functioning cells, the pheochrome cells of the adrenal medullary parenchyma. On the other hand, the causative factor of essential hypertension of the benign type is a matter of speculation, still resting on hypotheses, none of which is entirely tenable. In the second part of this paper (dealing with paroxysmal hypertension) a rather detailed consideration will be accorded the adrenal gland because it constitutes one of the many hypothetical etiological factors of essential hypertension, as well as being the actual site of the known causal pathology of paroxysmal hypertension.

Part I

Essential Hypertension of the Benign Type

Medical literature dealing with essential hypertension includes the life work of universally recognized specialists in all

* Received for publication July 27, 1939.

† A tentative figure based on incompletely classified forms of chronic cardiovascular disease encountered among 1105 autopsies performed by the author during his tours of duty.

branches of our profession. Almost all the valuable work now being accomplished on this subject is along medical, surgical and experimental chemical-physiological lines, and it would appear that too little attention is focussed on the data included in autopsy protocols.

The term essential hypertension has been generally accepted since Allbutt [1] in 1895 recognized the occurrence of isolated hypertension by following patients with increased blood pressure over periods of years. I would define essential hypertension as the manifestation of sustained high blood pressure without recognizable cause, although later secondary cardiac, cerebral and renal vascular changes occur. Naturally all individuals with essential hypertension of the benign type do not succumb to this ailment and the secondary vascular changes may merge into those of the senile form. Logically the term essential hypertension should be limited to the condition usually referred to as essential hypertension of the benign type.

In selecting the cases to be reported, 1 case essential hypertension of the benign type, the 2nd case one of paroxysmal hypertension, I was able to find records of 2 patients who presented much in common. Both were white males of the same age at the time of death (45 years); both were army officers, one a member of the Medical Corps, the other of the Veterinary Corps; and both died as the result of cerebral hemorrhage. I was enabled through the courtesy of the Office of the Surgeon General to secure the necessary data to prepare graphs illustrating the blood pressure readings of both officers from the time of their entry into the Service to death (Graphs 1 and 2).

Case 1. Essential Hypertension of the Benign Type

Clinical History: The patient, P. McN., was a white male, aged 45 years at the time of death. He was a moderate user of alcohol and smoked in excess, approximately 50 cigarettes daily. His father died at 60 years of age from cardiovascular disease with diabetes; his maternal grandmother died at 60 years from apoplexy; and a paternal aunt is reported to have had a blood pressure above 200 mm. Hg. for a number of years.

The past illnesses of the patient included mumps at the age of 22 years, complicated by orchitis. He had had mild influenza several times and several attacks of tonsillitis. The Wassermann and Kahn reactions were always negative.

In May, 1931, during the routine annual physical examination of this offi-

cer, a trace of albumin and hyaline casts were found in the urine. During 1932 the patient had repeated hematuria due to associated nephrolithiasis (see below). In December, 1932, he had severe epistaxis which required packing to control. The blood pressure readings are depicted in Graph 1.

The patient was admitted to the Walter Reed General Hospital, Washington, D. C., Nov. 14, 1933. At the time of admission he was ambulatory, afebrile and in fairly good general condition. The blood pressure on admission to the hospital was 220/140, and the pulse pressure 80 mm. Hg. The eyegrounds revealed marked angiosclerosis with scarring and recent small retinal hemorrhages. The blood count showed 3,830,000 erythrocytes; hemoglobin 75 per cent; total leukocyte count 8000; and the polymorphonuclears 69 per cent. The urea nitrogen was 20 mg. per cent, the blood sugar 108 mg. per cent. The basal metabolic rate was plus 2. The electrocardiogram showed left axis deviation and inverted T waves in lead I. Roentgenograms revealed widening of the aortic arch and left ventricular hypertrophy; the lungs were negative and the kidneys showed dense shadows in both renal areas. The specific gravity of the urine ranged from 1.006 to 1.027; all specimens of urine presented 1 to 2 plus albumin, sugar negative, many leukocytes and occasionally many erythrocytes. The urinary findings are in part accounted for by the complicating nephrolithiasis.

On Feb. 8, 1934, the patient complained of severe basal headache. On February 10 he had epistaxis. Muscular twitchings were noted and he became unconscious for one-half an hour. On February 11 the blood pressure was 270/170, and the pulse pressure 100 mm. Hg. A spinal puncture was made and clear fluid obtained. On February 23 at 12 midnight he had a convulsion and became unconscious for a short time. The blood pressure was then 235/143 mm. Hg. and flaccid paralysis of the right lower extremity supervened. A second convulsion accompanied by unconsciousness came on at 1.30 A.M. At 2.00 A.M. there was a third convulsion, and a fourth at 2.45 A.M. Flaccid paralysis of the right upper and lower extremities was noted. The left pupil was dilated and the right pupil was contracted to pin-point size. He remained unconscious until death, which occurred at 2.45 P.M., Feb. 24, 1934.

Postmortem Examination *

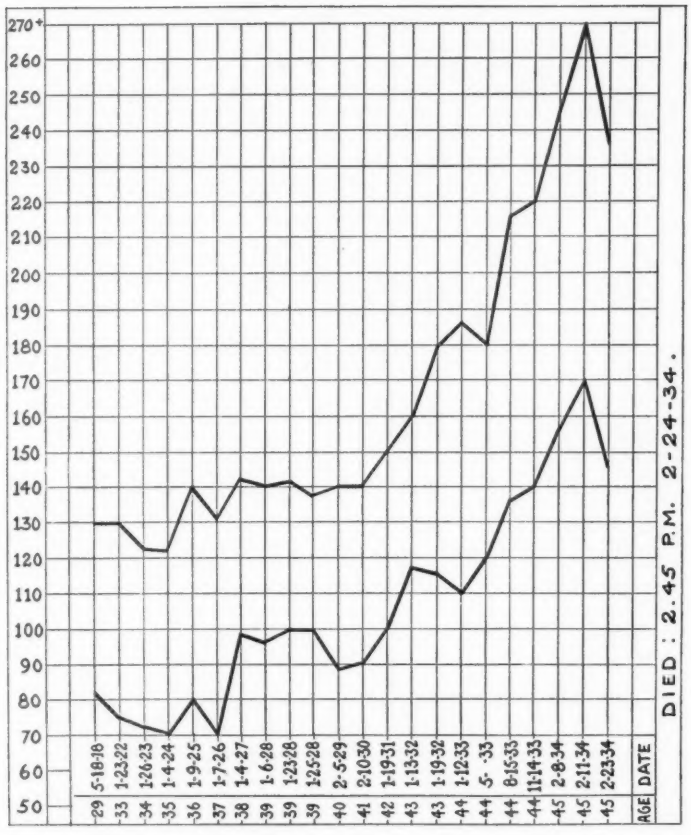
The body length was 175 cm., the weight approximately 61 Kg.

Heart: The heart weighed 550 gm. The wall of the right ventricle measured 7 mm. in thickness, the wall of left ventricle 2.8 cm. The aortic ring was 7.7 cm. in circumference. The coronary arteries showed moderate intimal thickening; the lumens were patent and the contents fluid blood.

Aorta: The aortic arch measured 6.5 cm. in circumference, the thoracic aorta 5.5 cm., and the abdominal aorta 4 cm. The aortic intima was diffusely thickened and presented plaques of fat imbibition, but no calcification or ulceration was observed.

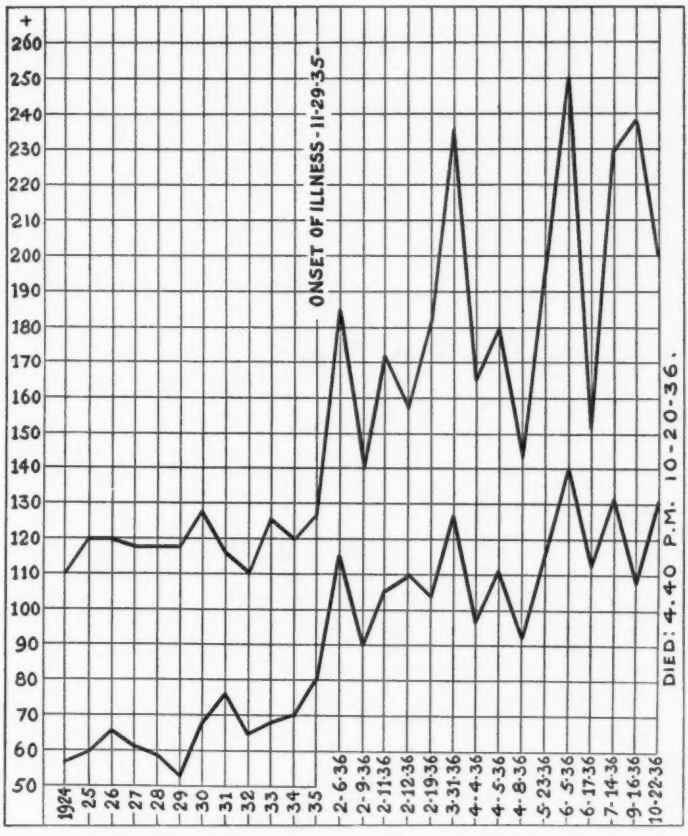
Adrenals: The adrenals were of normal size.

* A–1612, Walter Reed General Hospital, Washington, D. C.



GRAPH 1. Blood pressures, Case 1: essential hypertension of the benign type, death from cerebral accident (A–1612, W.R.G.H.).

The graph shows: (1) At no time was the pulse pressure within the normal limits indicated in Table I; from 1918, when the patient was 29 years old, to 1926 when he was 37 years old, the pulse pressures varied between 48 and 55 mm. Hg. (2) For a period of 3 years, 1926–1928 inclusive, the patient's diastolic pressure rose while the systolic pressure remained stationary and the pulse pressure dropped accordingly. (3) After 1929, when the patient was 40 years old, the diastolic and the systolic pressures rose concomitantly, fairly slowly at first, then with extreme rapidity up to the time of death which occurred Feb. 24, 1934.



GRAPH 2. Blood pressures, Case 2: paroxysmal hypertension, death from cerebral accident (A–2018, W.R.G.H.).

The graph indicates a normal blood pressure from the time of the patient's entry into the Service (1924) until the onset of the final illness, November, 1935, with the exception of a slight transitory rise during 1930 to 1931. After November, 1935, the blood pressure tracing resembles the temperature chart in a case of septicopyemia, marked by daily and hourly elevations and depressions. The final illness lasted approximately 11 months; death occurred Oct. 23, 1936 (the graph indicates the date of death as of Oct. 20, 1936, by error); cause of death, cerebral accident, hemorrhage into the optic radiation of the left hemisphere of the forebrain.

Kidneys: The right kidney weighed 110 gm., the left 120 gm.; combined renal weight 230 gm. The weight of the heart (550 gm.) divided by the combined renal weight (230 gm.) was 2.39 (ratio). The cortical surfaces of the kidneys were pinkish and granular; the renal cortices measured 5 mm. in thickness and the markings were preserved. The sinus fat was slightly increased. There were several mulberry-like calculi located in the calices of both kidneys and an impacted calculus was lodged in the lumen of the right ureter.

Microscopic examination of the renal tissue revealed moderately advanced nephrosclerosis of the benign type involving the interlobular and interlobar arteries. The afferent glomerular arterioles were not involved.

Brain: The brain weighed 1610 gm. The convolutions were flattened. The left lateral ventricle was distended with clotted blood due to a "blow-out" hemorrhage involving the posterior cornu of the left internal capsule with extensive spread through the adjacent parietal and frontal lobes of the forebrain and rupture into the left lateral ventricle. The hemorrhage was single. The vertebral arteries, the basilar artery, the circle of Willis and the cerebral branches all presented thickened parchment-like walls with encircling atheromatous bands.

Anatomical Diagnoses: (1) Eccentric hypertrophy of the heart, weight of heart 550 gm.; (2) atheromatosis of the aorta, moderately advanced; (3) coronary arteriosclerosis, moderately advanced without obstruction; (4) cerebral arteriosclerosis, advanced; (5) cerebral hemorrhage, massive, source of hemorrhage the left lenticulostriate artery, with destruction of the posterior cornu of the left internal capsule and rupture into the lumen of the left lateral ventricle of the brain; (6) renal arteriosclerosis, benign type, moderately advanced (microscopic finding); and (7) nephrolithiasis, bilateral, with impacted calculus in the lumen of the right ureter.

Comment

The blood pressure readings of the above case, from May 18, 1918, to death, were secured and are incorporated in Graph 1.*

* Through the courtesy of Major E. S. Cooley, U. S. Army, Office of the Surgeon General, Washington, D. C.

Study of the graph brings out the following points. (1) At no time was the patient's pulse pressure within the normal limits indicated in Table I. From 1918 when the officer was 29 years old, to 1926 when he was 37 years old, his pulse pressure varied between 48 and 55 mm. Hg. (2) For a period of 3 years, 1926 to 1928 inclusive, the patient's diastolic pressure rose while the systolic pressure remained stationary and the pulse pressure dropped accordingly. (3) After 1929, when the officer was 40 years old, the diastolic and the systolic pressures rose concomitantly, fairly slowly at first,

TABLE I

Data on Body Weight and Blood Pressure of 256 Army Officers

Decade	Number of cases	Body weight	Systolic blood pressure	Diastolic blood pressure	Pulse pressure
		lbs.	*mm. Hg.*	*mm. Hg.*	*mm. Hg.*
3rd	37	150	120	74	46
4th	51	160	122	77	45
5th	97	166	124	79	45
6th	64	161	132	81	51
7th	7	158	138	84	54
Averages:	256	159	127	79	48

NOTE: The ages vary from 24 to 64 years, the retirement age of army officers. The diastolic pressures present a fairly uniform rise with an increase of 10 mm. Hg. in 40 years, an average increase of 0.25 mm. Hg. per year, diastolic blood pressure.

then with extreme rapidity up to death, which occurred February 24, 1934.

The results of the annual physical examinations of 256 army officers for the year 1939 were made available for study * and are found to include valuable data relative to the blood pressure readings of a group of white males who are under constant medical supervision and whose lives and pursuits are fairly uniform. Of the total 256 officers 14, or 6 per cent, classify as obese, body weights averaging 90 Kg.; 6, or 2 per cent, present evidence of essential hypertension, not regarded as of sufficient severity to justify retirement; and 3, or 1 per cent, present some of the physical manifestations of the senile type of general arteriosclerosis. However, for the purpose intended, to supply workable averages, no cases were excluded and the body weights and the blood pressures of the 256 army officers are listed in Table I.

* Through the courtesy of Lt.-Colonel D. H. Mebane, Medical Corps, U. S. Army, Chief of the Medical Service at the Tripler General Hospital, Honolulu, Hawaii.

Part II

Paroxysmal Hypertension

The Adrenal Glands

In order to comprehend the pathology of the adrenal gland one must be reasonably cognizant of the histogenesis, anatomy and pathology of that organ.

Histogenesis: Quoting Maximow [2]: "The cortex develops from the celomic mesoderm on the medial side of the wolffian ridge, and the medulla from the ectodermal tissue from which the sympathetic ganglion cells also arise." Quoting Bailey and Cushing [3]: "The cells of the ganglionic crest . . . migrate into the visceral areas to form the anlage of the sympathetic nervous system. When they reach their final stations they differentiate into the neurons of the sympathetic ganglia, the capsular cells of these ganglia, and into the *chromaffine* cells of the adrenal medulla and analogous structures."

Anatomy: The adrenals are wedge shaped organs located on the upper poles of the kidneys. At birth the adrenals are relatively large, approximately one-third of the renal weight; during adult life the combined adrenal weight, ranging from 10 to 20 gm., is approximately one-thirtieth of the combined weight of the kidneys. The adrenals are located in a mesh of nerve fibers derived from the celiac ganglia, splanchnic, vagi and phrenic nerves. The adrenals receive their blood supply from three sources: from the inferior phrenic arteries, directly from the abdominal aorta, and from the renal arteries. The adrenal arteries break up into arterioles which course throughout the fibrous capsules of the adrenals; the adrenal arterioles reflect any degree of arteriolarsclerosis which may be found during the microscopic examination of the renal tissue. The arterioles in the adrenal capsules divide into capillaries which course inward between the lines of cortical cells to form the anastomosing capillary mesh of the formatio reticularis; the capillaries then reunite to form the sinusoids of the adrenal medulla. In the adrenal medulla the sinusoids join to form one large vein on either side, emptying into the inferior vena cava on the right and into the renal vein on the left, almost directly

below the eustachian valve of the heart. The walls of the two adrenal veins have a tunica media composed of longitudinally arranged fasciculi of smooth muscle fibers. In hypertension, chronic or due to any cause, the muscle fasciculi of the adrenal veins hypertrophy. Nervous stimuli to the adrenals pass: (1) to the chromaffin cells through the multipolar sympathetic neurons located in the substance of the adrenal medulla; and (2) to the longitudinal bundles of smooth muscle cells forming the walls of the adrenal veins, exciting a shortening of the veins with a pulling or suction action on the soft vascular tissue of the adrenal medulla, directly aiding the withdrawal of the secretory product (epinephrin) into the lumen of the sinusoids and veins, to be poured almost directly into the right atrium of the heart.

At birth the glomerular zone of the adrenal cortex is well developed; during adult life it gradually undergoes sclerosis, and at senility is hardly recognizable. Approaching senility the fascicular zone of the adrenal cortex undergoes nodular hyperplasia, at times quite marked though of no pathological significance. In essential hypertension the adrenal cortex is usually deep and the cells rich in lipoids. At birth the adrenal medullary parenchyma is sparsely represented though gradually increasing in relative volume as age advances. In cases of long continued hypertension from any cause the adrenal medullary parenchyma is increased in amount; the chromaffin cells show augmented physiological activity, manifested by the presence of numerous hyaloid cell inclusions located: (*a*) within the bodies of the large basophilic cuboidal and cylindrical cells palisading on the thin walled sinusoids of the adrenal medulla; and (*b*) within the capsules and in the bodies of the sympathetic neurons.

Physiology: The adrenal cortical parenchyma is indispensable for the continuance of life; adrenalectomized animals die after passing into a state of hemoconcentration with cationic imbalance; and there is marked increase in sodium elimination and potassium retention. Quoting Anderson and coworkers [4]: "Cushing's syndrome is an expression of the overproduction of cortin; studying the urine excretion of sodium and potassium and the blood level of these electrolytes, and comparing the findings with those of a patient with Addison's disease, it is reasoned that since some patients with Addison's disease are helped by a high sodium and

low potassium intake, then patients with Cushing's syndrome should be benefited by being placed on the opposite regimen, namely low sodium and high potassium intake." These authors state that this form of treatment proved effective in 1 case; the blood pressure decreased and there was a loss in weight.

Physiology of the Medulla: Epinephrin is the secretory product of the adrenal medullary parenchyma. The most delicate biological reaction, positive to a 1:800,000,000 concentration, fails to demonstrate any increase in the amount of epinephrin in the circulating blood. Quoting Rogoff and Marcus [5]: "Intravenously injected epinephrin disappears from the circulation so rapidly that it is rarely detected beyond the point of its pressor action and not after one complete circulation of the blood." The above observer believes that the concept that hypersecretion of epinephrin causes persistent elevation of blood pressure is untenable.

The normal physiological action of epinephrin may possibly be regarded as a direct action on the auriculoventricular node of the conductive system of the heart, located above the base of the septal cusp of the tricuspid valve. In paroxysmal hypertension resulting from adrenal medullary tumors of the pheochrome cell type the liberation of epinephrin during the episodes or crises exceeds all physiological and experimental bounds.

The correlation between the physiological functions of adrenal and thyroid is illustrated by 2 cases coming to autopsy. In 1 case, a white female,* aged 74 years, the thyroid weighed 55 gm., being five or six times the usual weight in senility, and it was rich in colloid. The adrenals presented complete bilateral destruction of the cortical parenchyma and most of the medullary portions by tuberculous caseous-nodose lesions. (2) In a 2nd case, a white male,† aged 48 years, there was complete replacement of the thyroid parenchyma by neoplastic tissue, carcinoma solidum. The combined weight of the adrenals was 45 gm., over twice the normal maximum weight of these organs. Another case,§ which illustrates the physiological function of the adrenal cortical parenchyma, was that of a white male, aged 39 years: the thyroid weighed 32 gm. (normal); the kidneys were polycystic, combined weight

* A–1802, Walter Reed General Hospital, Washington, D. C.
† A–1803, Walter Reed General Hospital, Washington, D. C.
§ A–2213, Walter Reed General Hospital, Washington, D. C.

7620 gm.; the combined adrenal weight was 26 gm., and the adrenals showed marked cortical hyperplasia regarded as the effort to maintain the cationic balance between the blood plasma and the blood and tissue cells of the patient.

Pathology: The pathology of the adrenal gland may be divided into: (*a*) lesions primary in the adrenal itself, cortical and medullary portions; and (*b*) lesions primary in the adrenal retroperitoneal rests, the organs of Zuckerkandl, and adrenal rests in the substance of the kidneys, the primary foci of Grawitz tumors. Adrenal cortical cell rests are sometimes encountered attached to the epididymes of the testes, but I have no record of primary tumors of adrenal tissue in this location. Limiting the discussion of adrenal neoplasia to that primary in the medullary parenchyma, the 1105 autopsies forming the basis of this study furnish two examples: (1) a neuroblastoma * having as its chief cell a unipolar neuroblast, a cell midway in its developmental position between the undifferentiated cells migrating from the ganglionic crest of the embryo and the highly differentiated chromaffin cells and sympathetic neurons of the adrenal medulla; and (2) a 2nd case,† a pheochromoblastoma, in which the chief cell was a variant of the highly differentiated chromaffin cells of the fully developed adrenal medullary parenchyma. As would be expected from their respective developmental positions, the neuroblastoma was highly malignant, widely metastasizing and without physiological activity, while the pheochromoblastoma was relatively benign, showing only local invasion and extension and presenting a very highly developed physiological activity manifested by episodes of hypertension of the paroxysmal type.

Abstracts of Reported Cases of Paroxysmal Hypertension

The medical literature includes reports of a number of cases of paroxysmal hypertension and the following case reports will be briefly abstracted.

Belt and Powell [6] described a syndrome characterized by paroxysmal hypertension and periodic tachycardia, pallor followed by a flushing of the skin, glycosuria, headache, nausea and vomiting, and pulmonary edema. The case reported is that of a Jewess aged

* A–1718, Walter Reed General Hospital, Washington, D. C.
† A–2018, Walter Reed General Hospital, Washington, D. C.

45 years. The patient died during a crisis accompanied by marked pulmonary edema. Autopsy revealed an adrenal tumor weighing 1000 gm. The chemical examination demonstrated 0.2 gm. of epinephrin per 100 gm. of tissue. The technic of the colorimetric estimation of epinephrin was that of Folin, Cannon and Denis.[7]

Labbé, Tinel and Doumer [8] report the case of a female, aged 28 years, who suffered from attacks of nausea and vomiting, three to four attacks a week. During these attacks the systolic blood pressure rose to 280 mm. Hg. Glycosuria was noted. The hypertension varied from day to day and from hour to hour. Pulmonary edema developed and the patient died. The left adrenal was replaced by a tumor mass about 6 cm. in diameter.

Labbé, Violle and Azérad [9] report a case of "paroxysmal sympatheticotonia" in a male patient aged 29 years, who died as the result of cerebral hemorrhage.

Mayo [10] reports the case of a female aged 30 years. The chief symptoms were nausea and vomiting, and cough with blood tinged expectoration. The crises were marked by paroxysmal hypertension during which the systolic blood pressure varied between 300 and 180 mm. Hg. The patient was operated upon and a pheochrome tumor (paraganglioma) was removed. The tumor mass measured 6 by 4 cm. and was located retroperitoneally mesial to the left kidney. The patient made a complete recovery.

Rabin [11] reports the case of a Polish woman aged 45 years. The clinical course was marked by episodes characterized by nausea and vomiting with rise in blood pressure up to 226/180 mm. Hg. Coma developed and the patient died. At autopsy an adrenal medullary pheochromocytoma was found which yielded 1.5 mg. of epinephrin per gm. of tissue.

Burgess, Waterman and Cutts [12] report the case of a Jewess, aged 25 years, who complained of palpitation associated with pallor and constipation. At the onset of the crises the pulse rose to 144 per minute; bradycardia accompanied by extrasystoles developed. Headache was severe. The pyelogram demonstrated a downward displacement of the right kidney. The blood sugar was 125 mg. per cent, and the non-protein nitrogen 31 mg. per cent. The Wassermann test was negative. The patient was operated upon and died almost immediately from pulmonary edema. During operation a tumor mass 9 cm. in diameter, soft and hemorrhagic,

was removed from the upper pole of the right kidney. The microscopic diagnosis was pheochromocytoma primary in the medulla of the right adrenal. No metastases were found.

Geschickter [13] reports a "benign paraganglioma" of suprarenal medullary tissue in a woman aged 53 years. Geschickter states: "In paragangliomas of the suprarenal glands, hypertension, hypotension, and vasomotor instability are not infrequently observed . . . the suprarenal cortex may be invaded and destroyed. . . ."

Ewing [14] classifies adrenal tumors as cortical and medullary. Medullary tumors of the adrenal are subgrouped as focal hyperplasia, neuroma ganglionare, neuroblastoma, and chromaffin cell tumors. He states that medullary chromaffin cell tumors form large masses. The sinuses are invaded but there are no metastases. The structure is composed of groups of round, oval and polygonal cells, most of which are small. Giant cells with single or multiple nuclei occur. Pigment is irregular in distribution. Homogeneous acidophilic cell inclusions are usually present.

Shipley [15] reports the case of a young married woman, aged 26 years, who complained of attacks characterized by cardiac palpitation, nausea and vomiting. Severe occipital headache was an increasingly troublesome symptom. Her blood pressure varied from 120/90 on admission, to 190/98, rising quickly to 219/110 and falling rapidly to 176/76 mm. Hg. The diagnosis of adrenal tumor was made but the side was not determined. The patient was operated upon, first on the left side, and the left adrenal was found to be normal; on the right side a tumor mass primary in the right adrenal was discovered and removed. The day following the second operation the patient improved. During convalescence the blood pressure remained low, 116/74 mm. Hg. Ten months later she reported herself to be free from previous symptoms. The mass removed weighed 115 gm. A small amount of cortical adrenal tissue remained extending over one side of the neoplasm. Microscopic sections showed the tumor to be composed of oval and polygonal cells arranged in small alveoli separated by capillaries. The diagnosis was paraganglioma.

In my opinion the term paraganglioma should be limited to tumors arising from retroperitoneal rests of adrenal medullary tissue, while neoplasia primary in the adrenal medullary parenchyma should be designated as pheochromocytoma or pheochromo-

blastoma, depending on the degree of differentiation manifested by the tumor cells.

Kelly and his associates [16] suggest the following reasons for the paroxysmal nature of the hypertension associated with pheochrome cell tumors of adrenal medullary tissue: (*a*) periodic hemorrhages into the substance of the tumor mass; (*b*) any unusual exertion, such as bending, expressing epinephrin from the neoplastic tissue; and (*c*) emotional reactions such as are believed to stimulate an increased output of epinephrin. They are of the opinion that the complete absence of signs of arteriosclerosis is of particular interest; this is evidence against the conception that epinephrin plays any part in the production of arteriosclerosis and essential hypertension. I do not entirely concur with the foregoing assumption; although epinephrin probably does not play a primary rôle in essential hypertension, still I believe that a secondary physiological function is maintained by the adrenal medullary parenchyma in essential hypertension. They report the case of a married woman, aged 37 years, who complained of episodes during which the blood pressure rose from 90/70 to 280/160 mm. Hg. During these episodes extreme cutaneous pallor developed and the patient was nauseated. The attacks lasted about 15 minutes. The pulse was not appreciably accelerated. The blood sugar was 118 mg. per cent. Indefinite resistance and tenderness were elicited above the right kidney, which was palpable. The patient was operated upon and a tumor mass was located cephalad to the upper pole of the kidney. The mass was 10.5 cm. in diameter and weighed 240 gm. On chemical examination the neoplastic tissue yielded approximately 300 mg. of crystalline epinephrin. Postoperatively the convalescence was uneventful and no further episodes occurred.

Evans [17] reports the case of a girl, aged 12 years, who died during an episode of paroxysmal hypertension. Autopsy revealed a pheochrome cell tumor primary in the medullary portion of the left adrenal.

Wells and Boman [18] note that the characteristic symptoms of 13 patients completely disappeared following surgical removal of benign tumors composed of epinephrin-producing cells such as are normally present in the adrenal medulla. They state that during severe attacks there may be failure of the left ventricle of the heart, manifested by cyanosis, coughing with blood tinged sputum,

and that the sudden transient spasm of the arterioles of the body accounts for many of the manifestations of this condition. The attacks tend to increase in severity and frequency. They report the case of a female, a school teacher aged 30 years. The initial symptom was a pounding of the heart like a sledge hammer. During these attacks the blood pressure rose to 180/140 mm. Hg. and then dropped suddenly. The patient died during anesthesia for attempted appendectomy. Autopsy demonstrated a tumor primary in the right adrenal and weighing 20 gm. Chemical examination of the neoplastic tissue showed 1 per cent of epinephrin.

The 12 cases of pheochrome cell tumors, including the following case, may be classified as follows: females, 10 (2 Jewesses); males, 2; average age 34 years. Two of the tumors were primary in retroperitoneal rests of adrenal medullary tissue (paragangliomas); 5 were primary in the right adrenal medulla and 2 in the left; in 3 of the cases abstracted the side involved is not indicated. In 3 cases transient glycosuria was noted. The 2 male patients died as the result of cerebral hemorrhage. Three of the patients died during crises as the result of marked pulmonary congestion and edema. Four of the female patients were operated upon; 1 died immediately following operation, and 3 made complete postoperative recovery without subsequent episodes.

Case 2. Paroxysmal Hypertension

Clinical History: The patient, A. D. M., was a white male, a veterinarian of the Medical Department of the U. S. Army. There were no previous illnesses other than the usual diseases of childhood. He used no alcohol and smoked in moderation. The family history revealed no chronic cardiovascular disease. The patient was admitted to the hospital Nov. 15, 1929, stating that he had passed a large tarry bowel movement. On admission to the hospital he was pale and anemic in appearance. The blood pressure was 108/60 mm. Hg. He was treated on the basis of an emergency duodenal ulcer and was discharged on March 17, 1930.

The patient had enjoyed good health from March, 1930, to November, 1935. On Nov. 29, 1935 he caught cold, complained of headache and was nauseated. He was hospitalized, placed on the Sippy regimen, and discharged later improved. On December 7th he suddenly felt cold and his head ached. Within a few hours he became delirious for about 48 hours, and after complete consciousness returned he remarked that his vision had become hazy. The blood counts, the Wassermann reaction, the spinal fluid examinations and the results of blood chemistry were all negative. The blood pressure on Jan. 5, 1936 was 172/118 mm. Hg.

The patient was transferred to the Walter Reed General Hospital, Washington, D. C., on Feb. 4, 1936. At this time he was 45 years old. On admission

the pupils were equal and reacted to light and accommodation. The blood pressure was 154/96 mm. Hg. On February 7th, after an enema, the patient became nauseated and complained of severe headache; following catharsis (castor oil) the headache subsided and he felt better. On March 4th nausea and vomiting recurred accompanied by severe headache. A spinal tap was made; the spinal fluid was clear, pressure 20 mm. Hg. A brain tumor was considered as a possibility. On March 16th the urine became bloody; the clinical record states that "hypernephroma must be considered." On March 19th the urine was free from blood. On March 23rd the case was considered as one of "undoubtedly essential hypertension with renal arteriolarsclerosis." On March 27th hematuria occurred again and epididymitis (left) developed. By April 18th the patient's condition improved; he had gained in weight and was out of bed in a wheel-chair. On May 8th the headaches returned and became increasingly severe; he was nauseated and vomited frequently. The clinical record on June 5th notes: "The patient remarked that he felt better after saline infusions." On July 13th another crisis developed; the clinical notes state: "The distressing spells are coming on more frequently; the attacks arise when the patient is constipated and are initiated by headache, nausea and vomiting. The subjective symptoms are relieved by saline infusions and by castor oil. During the attacks the extremities are cold." On October 22nd an attack began; the patient vomited and became restless and irrational. There was involuntary urination. The blood pressure registered 200/130 mm. Hg. The urea nitrogen was 15 mg. per cent. The patient closed his right eye with difficulty. The next day right hemiplegia developed; the pulse became feeble and the respirations stertorous. The patient died at 4.40 P.M. on Oct. 23, 1936. The duration of the final illness was approximately 11 months.

The variations of the blood pressure readings are depicted in Graph 2. Throughout the final illness there was no elevation of temperature except during the last 2 days when the temperature rose to 102° F. The pulse varied between 80 and 110 beats per minute. At no time during the final illness were abdominal pain and tenderness elicited.

Laboratory Data: A roentgenogram on Feb. 6, 1936, showed that the cardiac shadow was normal in size, shape and position; the lungs were essentially normal. On August 31st flat plates of the abdomen were negative. No pyelograms were made. Some of the specimens of urine revealed 2 plus albumin, sugar negative, many leukocytes and erythrocytes, but no casts. The blood count on February 5th revealed: erythrocytes 3,960,000; hemoglobin 70 per cent; total leukocyte count 10,000; polymorphonuclears 68 per cent; eosinophils 3 per cent.

Postmortem Examination *

The body was that of a poorly nourished white male, aged 45 years. The body length was 176 cm., the weight approximately 55 Kg. The voluntary musculature was fairly well developed. The pupils were equal at 3 mm. The neck and chest were of normal contour. The anterior abdominal wall was flat. No edema of the feet or ankles was present.

* A–2018, Walter Reed General Hospital, Washington, D. C.

Thyroid: The thyroid weighed 29 gm. On microscopic examination the acini were large and the colloid homogeneous. The epithelium was of the cuboidal and flat types. There was no lymphocytic reaction. No thymus rest was encountered.

Lungs: Microscopic examination of the pulmonary tissue revealed an acute bronchiolitis and peribronchiolitis.

Heart: The heart weighed 385 gm.; the subepicardial fat was scant in amount. The wall of the left ventricle measured 2.2 to 1.5 cm. in thickness. The aortic ring was 7.3 cm. in circumference; the mitral ring 10 cm. The valves were normal. The coronary arteries and the aorta presented moderate intimal thickening, but no lime salt deposits or ulceration were present. The thoracic aorta measured 5.3 cm. in circumference and the abdominal aorta 4 cm.

Stomach and Intestines: Normal in appearance. The colon contained large masses of hard fecal material.

Spleen: The spleen was small, weighing 50 gm.

Adrenals: The left adrenal weighed 7 gm. and was normal in size and contour. The retroperitoneal fat surrounding the left adrenal included small aberrant groups of adrenal cortical parenchyma. On microscopic examination the cells of the left adrenal cortex were rich in lipoids and the medullary portion showed no alteration from the normal.

Tumor Mass Replacing the Right Adrenal: A tumor mass, 12 by 9 cm. in diameter, weighing approximately 200 gm. (section of the inferior vena cava is included in the gross specimen illustrated in Figure 1) was present in the right adrenal. The mass shelled out easily from the surrounding fat but was firmly united to the wall of the inferior vena cava and to the thickened overlying parietal peritoneum. Neoplastic tissue appeared in the lumen of the cava in the form of a nodule about 0.5 by 2 cm. The outer surface of the tumor was coarsely nodular, the consistence firm, and the cut surfaces brownish gray. After remaining in Kaiserling I the neoplastic tissue assumed a darker brown hue. The free surface of the parietal peritoneum overlying the tumor mass presented innumerable nodules varying in size up to 0.25 cm. The cut surfaces of the peritoneal nodules had the same gross appearance as the cut surfaces of the primary growth. Similar nodules were found on the under surface of the right leaf of the diaphragm

cephalad to the right adrenal tumor mass, and attached to the free surfaces of both leaves of the mesentery of the small intestine.

Microscopic Examination of the Newgrowth: The growth is very cellular; the cells are arranged in groups (alveoli), round to oval in contour, and are located in close proximity to thin walled blood channels (Figs. 2–4). The cells are quite pleomorphic, fusiform, cylindrical and pear shaped, and in some instances are united to the supporting fibrillary scaffolding by stem-like protoplasmic processes. The cells vary greatly in size and shape. The cytoplasm of the cells is amphophilic and finely granular; some of the cells contain unstained vacuoles and in other cells there are acidophilic hyaloid inclusions. The nuclei of the tumor cells are mostly small, circular in outline and eccentric in position; the linin network is poorly differentiated. A few cells present larger oval, hyperchromatic nuclei and scattered cells have two or more nuclei. Many of the neoplastic cells present an atypical nuclear division; the nuclear chromatin is divided into from 6 to 50 hyperchromatic bodies, oval, rod and dumbbell shaped and massed in the central zones of the dividing nuclei. In scattered areas throughout the newgrowth the neoplastic tissue contains a dull brown, coarsely granular pigment; the pigment gives a negative iron reaction with potassium ferrocyanide and is consequently regarded as melanin. In sections stained by Bielschowsky's method the fibrillar stroma includes no argentaffin fibrils; the tumor cells are silver-positive, and the cytoplasm of the cells appears as a fine silver gray dust including coarser particles of reduced silver.

Kidneys: The right kidney weighed 145 gm., the left 140 gm.; combined renal weight 285 gm. The weight of the heart (385 gm.) divided by the combined renal weight (285 gm.) equals 1.35, as compared with the cardiorenal ratio of 2.39 in the case of essential hypertension reported above.

Urinary Bladder: The wall was thickened and the trabeculae prominent. No cellule formation was present. The bladder mucosa was studded with innumerable pale gray, slightly raised points the size of the head of a common pin. The trigone was low and narrow and the ureteral orifices normal. The microscopic picture presented by the bladder mucosa indicated a chronic follicular cystitis.

Left Testis: The epididymis was thickened and indurated and cross sections revealed numerous small abscesses.

Brain: The brain weighed 1440 gm. The contour was normal and the pia normal. Sectioning the brain showed an area of recent hemorrhage about 2 cm. in diameter located in the optic radiation of the left hemisphere of the forebrain. The hemorrhage was surrounded by a wide zone of edema with innumerable points of secondary capillary bleeding.

Pituitary: The pituitary was normal in size and contour. The basilar artery, the circle of Willis and the cerebral arteries presented normal walls. The contents were fluid blood.

Anatomical Diagnoses: (1) Chromaffin cell tumor (pheochromoblastoma) primary in the medullary portion of the right adrenal; neoplastic invasion of the lumen of the inferior vena cava; implantation metastases on the free surface of the overlying parietal peritoneum and on the contiguous peritoneal surface of the right leaf of the diaphragm; small implantation metastases on the free surfaces of both leaves of the mesentery of the small intestine, but no other distant metastases. (2) Cerebral hemorrhage, single, optic radiation of the left hemisphere of the forebrain. (3) Cystitis (urinary bladder), chronic, follicular; and (4) epididymitis, subacute, suppurative (left testis).

Comment

The blood pressures in the above case are charted from 1924 to 1936 inclusive and are incorporated in Graph 2. The graph indicates that during 1930 there was a slight rise in both the systolic and the diastolic readings. The initial hospitalization of the patient was Nov. 15, 1929; the blood pressure of that date was 108/60 mm. Hg. From the onset of the final illness, Nov. 29, 1935, to death, which occurred Oct. 23, 1936, there were abrupt alterations in the blood pressure marked by daily and hourly changes, making the blood pressure graph resemble a temperature chart in a case of septicopyemia. The final illness was subacute in nature and the duration was approximately 11 months.

In explanation of the constipation and headache, two of the more prominent and distressing subjective manifestations, Wiggers,[19] in referring to the effect of epinephrin on muscle, writes that excitatory effects result on the pyloric and ileocolic sphincters.

Inhibition of contractions and tonus occurs in the gastrointestinal tract. In reference to headache, he states that increase in intraventricular pressure is believed to be the most common cause of headache. Intracranial and intraventricular pressure are simultaneously increased as a result of an increase in arterial pressure or reflex dilatation of the cerebral vessels. The headache so frequently associated with great hypertension becomes explicable on the grounds that increased arterial pressure *per se* increases brain volume and perhaps also increases the secretion of cerebrospinal fluid by the choroid plexus.

The episodes characterizing cases of paroxysmal hypertension associated with pheochrome cell tumors primary in the adrenal medullary parenchyma are, in my opinion, wholly explicable on the ground of the accumulation, in the substance of the newgrowth, of the secretory product (epinephrin) of the tumor cells. In any newgrowth there is not the normal physiological nerve control of the parenchyma and the normal mechanical removal of the secretory products by sinusoids is lacking; therefore the secretory products (if such be formed) will accumulate until large amounts gather and are "spilled over" into the capillary system of the neoplasia.

Almost all of the case reports illustrating paroxysmal hypertension (including that of the author) note some degree of tachycardia; some of the case reports indicate bradycardia. The pulse rate, in my opinion, indicates the amount of epinephrin "spilled over" during the episodes. (1) If the amount of epinephrin liberated be very great, sufficient to escape complete oxidation during its passage through the lungs, bradycardia will result. (2) If the amount of epinephrin liberated during the episodes be entirely or almost completely oxidized during its passage through the pulmonary circulation, tachycardia will result. Marey's law, quoted from Howell,[20] reads as follows: "Marey (La Circulation du Sang, Paris, 1881) gives the two following laws: (1) Whatever increases or diminishes the *force* with which blood is driven from the heart toward the periphery will cause the velocity of the blood and the pressure in the arteries to vary in the same sense. (2) Whatever increases or diminishes the ***resistance*** offered to the blood in passing from the arteries to the veins will cause the velocity and the arterial pressure to vary in an inverse sense. That is, an increased

resistance diminishes the velocity in the arteries while increasing the pressure, and *vice versa*." The slowing of the pulse after the intravenous administration of adrenalin is probably accounted for by secondary reflex vagus excitation because it does not occur (experimentally) after preliminary vagus section.

Starling [21] states: "The point of attack of adrenalin appears to be in the muscular or glandular tissue, since its effects may not only be obtained after destruction of the cord and sympathetic plexuses, but even obtained (and in an exaggerated degree) after time has been allowed for the peripheral (post-ganglionic) fibers to degenerate as a result of extirpation of the corresponding ganglia."

Referring to the transient glycosuria noted during or immediately after episodes of paroxysmal hypertension, I believe the glycosuria to be strictly renal in type, due to the sudden increase in pressure in the "arterial mirabile" of the kidneys (glomerular tufts of the renal corpuscles, including the afferent and efferent glomerular arterioles). Starling states that adrenalin acts directly on the liver cells, inciting a rapid discharge of glycogen with resulting hyperglycemia and glycosuria. In the 12 cases of paroxysmal hypertension cited, transient glycosuria was noted only in 3, and the highest blood sugar estimation was 125 mg. per cent, within the normal limits.

Summary and Conclusions

The main object of the foregoing discussion of essential hypertension and paroxysmal hypertension, with reports of contrasting cases, is to assist in the differential diagnosis between two conditions which may be said to be similar yet entirely different from one another. The early recognition of the physiological response to pheochrome cell tumors primary in the adrenal medullary parenchyma is of great therapeutic importance by reason of the complete cure afforded by surgical intervention in a fairly high percentage of cases, contrasting with the poor prognosis offerable individuals with essential hypertension even of the benign type.

The following are the more apparent differential points dis-

tinguishing the two conditions (essential and paroxysmal hypertension) as exemplified by the case reports:

	Essential Hypertension	Paroxysmal Hypertension
(*a*) Family history	Often strongly positive for chronic cardiovascular disease	Usually negative
(*b*) Previous illness	Previous hospitalization for cardiovascular disease may be listed	Usually irrelevant
(*c*) Present illness	Marked chronicity	Course relatively short and marked by episodes increasing in frequency and severity
(*d*) Special examinations		
X-Ray	Left ventricular cardiac hypertrophy. The aortic arch may be widened	Heart not necessarily enlarged; aorta may be normal
Electrocardiogram	Left axis deviation and T wave negativity may be noted	Not necessarily altered from normal except during episodes
Blood pressure	See Graph 1	See Graph 2
Pyelogram	Negative	Downward displacement of kidney on the side of the adrenal tumor mass may be noted
Blood counts	Slight secondary anemia	May be normal
Urine examinations	Traces of albumin with hyaline casts may be reported	Normal except during episodes when there may be transient renal glycosuria
Retinoscopy	Marked retinal angiosclerosis with hemorrhages	Retinal hemorrhages may be noted
(*e*) Causes of death	Cerebral accident; coronary accident; uremia; intercurrent disease. Death usually during the fifth or sixth decade of life	Cerebral accident which may be atypical in location. Congestive heart failure (left ventricular failure). Death during the fourth decade of life, at 34 years average of cases listed

Other conditions which must be ruled out in arriving at the recognition of pheochrome cell tumor primary in the adrenal medullary parenchyma include: (1) Hypertension associated with renal arteriolarsclerosis, the so-called malignant phase of essential hypertension; (2) renal hypertension associated with the secondary contracted kidneys seen in chronic glomerular nephritis; (3) congenital and acquired forms of renal maldevelopment, in-

cluding polycystic kidneys; (4) neoplastic hyperplasia of the adrenal cortical parenchyma (hypernephroma and Grawitz tumors); and (5) brain tumors.

The reported case of paroxysmal hypertension was not diagnosed ante mortem, having been overlooked by competent observers; however, the consolation remains that the pheochromoblastoma found at autopsy was quite inoperable, due to the fact that (see Figure 1) it had invaded the lumen of the inferior vena cava and was not entirely benign in character, innumerable small implantation metastases already having made their appearance.

In the foregoing article the subject of essential hypertension has hardly been touched upon and it is my intention, in a series of subsequent articles, to report contrasting cases of essential hypertension of the so-called malignant type and nephritic hypertension, and to discuss and illustrate the more commonly encountered syphilitic cardiovascular reactions found at autopsy.

NOTE: I wish to express my appreciation to the Curator of the Army Medical Museum, Washington, D. C., for his assistance in the necessary microphotography.

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DESCRIPTION OF PLATES

PLATE 110

FIG. 1. Pheochromoblastoma primary in the medullary portion of the right adrenal (Case 2, A–2018 W.R.G.H.). Posterior aspect of the tumor mass. The inferior vena cava is opened longitudinally. The growth invades the lumen of the cava in the form of an ovoid nodule about 0.5 by 2 cm. The neoplastic tissue was firm in consistence, the cut surfaces brownish gray, changing to a darker brown after having been immersed in Kaiserling I.

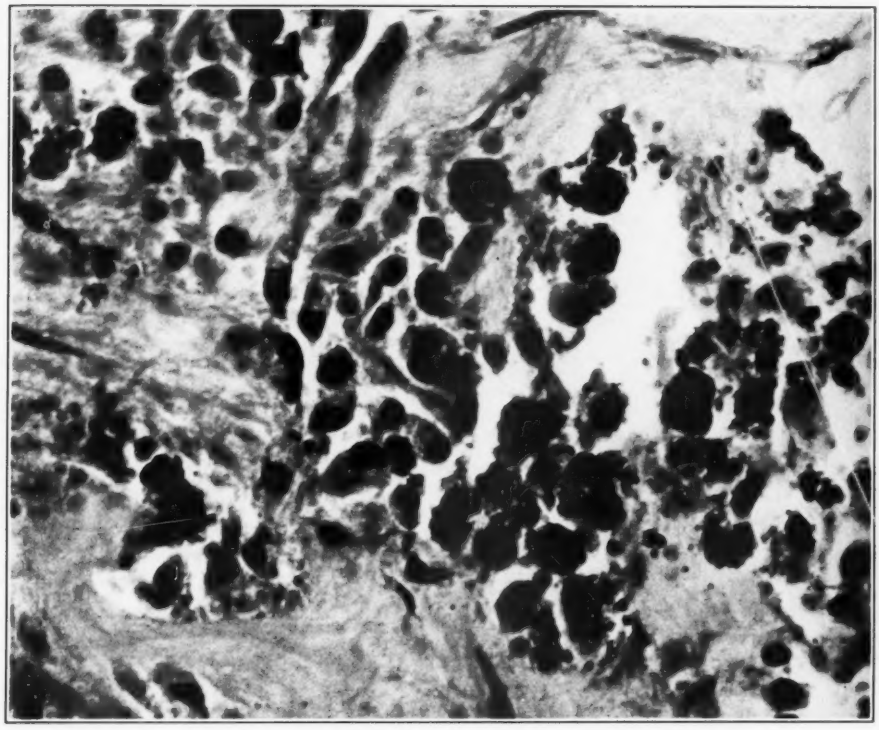
1

Foucar Essential and Paroxysmal Hypertension

PLATE III

FIG. 2. Pheochromoblastoma primary in the medullary portion of the right adrenal (Case 2). Microphotograph showing one of the scattered collections of iron-free pigment regarded as melanin. Hematoxylin-eosin stain. × 800.

FIG. 3. Pheochromoblastoma primary in the medullary portion of the right adrenal (Case 2). Microphotograph showing the irregularity in the size of the nuclei, the poorly differentiated nuclear detail, the tendency to eccentricity in nuclear placement, and the stippling of the cytoplasm of some of the cells. Hematoxylin-eosin stain. × 810.



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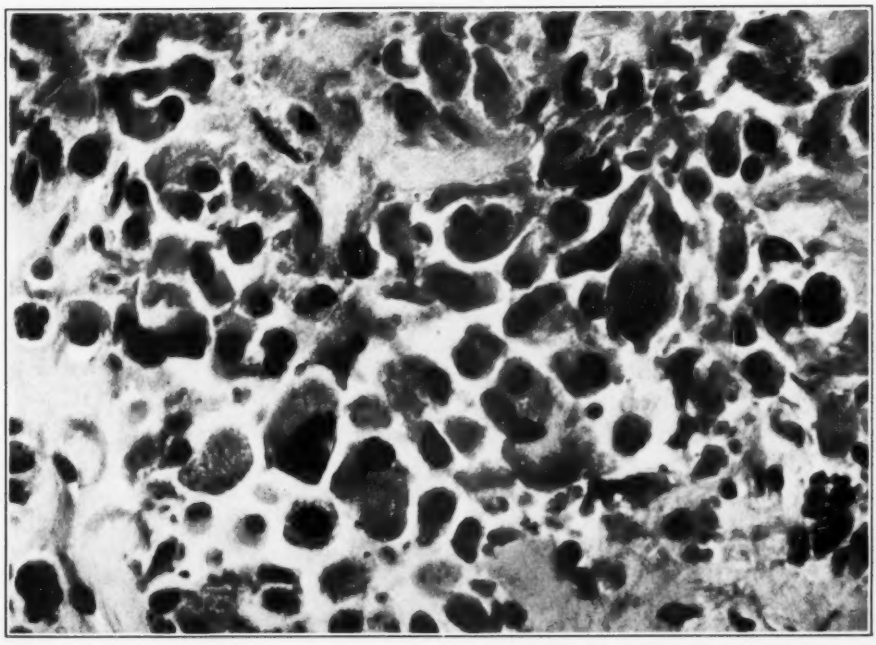

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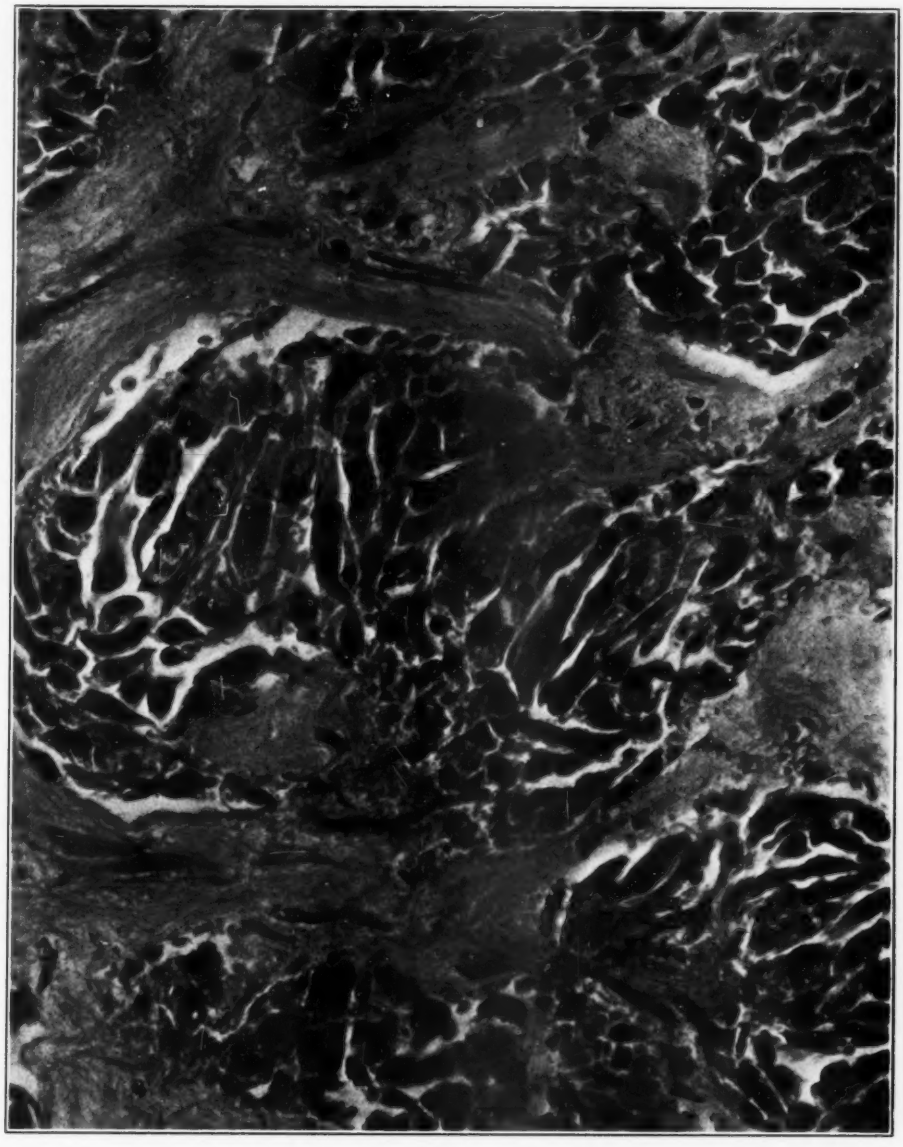
PLATE 112

FIG. 4. Pheochromoblastoma primary in the medullary portion of the right adrenal (Case 2). Microphotograph showing the alveolar arrangement of the tumor cells. The nuclei of the cells are hyperchromatic and the nuclear details are poorly differentiated. The interstitial stroma consists of delicate collagenous fibrils, glial-like in appearance. Hematoxylin-eosin stain. × 850.



4

Foucar Essential and Paroxysmal Hypertension

IRON HEMATOXYLINS CONTAINING FERRIC AND FERROUS IRON *

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Following our previous studies [1] on the superior keeping qualities of Janssens' iron hematoxylin and the demonstration of the preservation of the blue iron lake of hematoxylin by addition of a ferrous salt to the Janssens' formula, it seemed necessary to explore further the optimum concentrations and proportions of the various components of the mixture.

Experiment I was designed to compare the availability of ferrous sulphate in place of the ferrous ammonium sulphate used by Earle.[2]

TABLE I

Composition of Solutions in Experiment I

Solution		I–1	I–2	I–3	I–4	I–5	I–6
(A) Ferric ammonium sulphate		1.0	2.5	10.0	1.0	2.5	10.0 *gm.*
Ferrous ammonium sulphate		1.0	2.5	10.0	..	..	.. *gm.*
Ferrous sulphate		..	..	..	1.0	2.5	10.0 *gm.*
Distilled water		100	100	100	100	100	100 *cc.*
(B) Hematoxylin	1 gm.	Solutions A and B were mixed on August 8, 1938.					
Alcohol, 95%	50 cc.						
Glycerine, C P	50 cc.						

Six solutions were used, the 1st containing 1 gm. each of ferric ammonium citrate and of ferrous ammonium citrate in 100 cc. of distilled water, and 1 gm. of hematoxylin in 50 cc. each of glycerine and of alcohol. In the 2nd solution the iron salts were increased to 2.5 gm. each, and in the 3rd to 10 gm. each. In the 4th, 5th and 6th solutions ferrous sulphate was substituted for ferrous ammonium sulphate in 1, 2.5 and 10 gm. quantities respectively. These solutions were made up on August 8, 1938.

All solutions remained blue-violet for at least 24 hours, but by August 10 the 4 weaker solutions had turned brown. Staining with the freshly prepared solutions gave sharp nuclear staining in 5

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minutes with all solutions, but with Solutions 3 and 6, those containing the most iron, there was less staining of other tissue elements. Staining with Solutions 3 and 6 was still excellent after 8 days and 40 days. In about 6 weeks Solution 3 had lost its blue-violet color, becoming purple, and after 3 months, purplish brown. At this time Solution 6 was still violet. Work was interrupted from this time until July, 1939, the solutions remaining closed with cork stoppers on a window sill over a steam radiator. In July, 1939, all solutions were brown, but Solution 6 still gave fair nuclear staining.

It appeared that ferrous sulphate could be substituted for ferrous ammonium sulphate and that fairly high salt concentrations were necessary for stability of the solutions. The superiority of the simple ferrous sulphate solution is not improbably due to the higher iron content of the 10 gm. of the salts used (2.01 versus 1.42 gm.). The ferric iron content was 1.16 gm.

Experiment II was designed to test the proper proportion of the hematoxylin to the total iron salts. A solution of ferric ammonium sulphate, 17.27 gm., and ferrous sulphate, 9.96 gm. per 100 cc. of water, was made up. This gives 2 gm. each of ferric and ferrous iron as Fe. This iron solution was mixed with varying amounts of distilled water to make 20 cc. quantities, and 20 cc. of 1 per cent hematoxylin in glycerine and alcohol was added on August 9, 1938.

Solution 1 gave satisfactory staining in 5 minutes, Solutions 2 to 9 overstained in 5 minutes, Solutions 10 to 14 gave fully satisfactory staining in 5 minutes, and in 30 minutes Solutions 10 and 11 gave slight overstaining, and Solutions 12, 13 and 14 fully satisfactory staining.

All solutions were blue-violet when made up; in 1 day the first 3 were brown, in 1 week 2 more, in 5 weeks the first 7 were brown and the last 4 violet. In 3 months the last 2 were purple, the rest purplish to yellowish brown, and by July, 1939, all were brown with a little pinkish tinge in 13 and 14. Staining on July 26, 1939 showed Solutions 13 and 14 fully satisfactory, 12 good, and 9, 10 and 11 fair.

This test indicated that relatively high concentrations of the iron salts in relation to the amount of hematoxylin are necessary for stability.

Adopting a level of 1.75 gm. of ferric iron as adequate from

TABLE II

Schedule of Dilutions and Iron Content of Solutions in Experiment II

Solution	II–1	II–2	II–3	II–4	II–5	II–6	II–7	II–8	II–9	II–10	II–11	II–12	II–13	II–14
Ferric ferrous iron solution	1	2	3	4	5	6	7	8	9	10	12.5	15	17.5	20
Distilled water	19	18	17	16	15	14	13	12	11	10	7.5	5	2.5	0
Gm. of Fe^{++} and of Fe^{+++} per 100 cc.	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.25	1.5	1.75	2.0

Experiment II, Experiment III was designed to test the proper amount of ferrous iron.

The following solutions were used to make each test solution: (1) A solution of 15.12 gm. of ferric ammonium sulphate in 52 cc. of distilled water. (2) A solution of 19.92 gm. of ferrous sulphate in 48 cc. of distilled water, or a part of this solution and enough additional water to make 48 cc. (3) A solution of 1 gm. of hematoxylin in 50 cc. each of glycerine and alcohol. Each solution of a final volume of 200 cc. contained 1.75 gm. of ferric iron, 1 gm. of hematoxylin and a quantity of ferrous iron varying from none to 4 gm., so that Solution A contained 0.1 gm., B, 0.2, and so on to J, 1 gm., K, 1.2 gm., and so on to O, 2 gm., P, 2.5, Q, 3, R, 3.5 and S, 4 gm. T lacked ferrous iron. Mixtures were made on August 10, 1938, and all were blue-violet when made. In 5 hours T was brown, A and B, purplish brown. By the next day all solutions with 0.8 gm. or less of ferrous iron were brown, those with 0.9 and 1, purplish brown, and the rest violet or blue-violet. In a week solutions with 1.8 gm. or more ferrous iron were still violet or blue-violet, in a month 2 gm. was the lower limit of violet, with little further change in 3 months. In July, 1939, solutions with 3, 3.5 and 4 gm. of ferrous iron were still distinctly purplish.

On the 7th day solutions with 1.8 gm. and more of ferrous iron gave optimal staining either in 5 or in 30 minutes. The control with no ferrous iron was distinctly poor, better in 30 minutes than in 5, and solutions with about 0.5 to 1 gm. ferrous iron were good in 5 minutes, optimal in 30. After 11½ months the last 3 solutions still gave excellent staining either in 5 or in 30 minutes, Solution P with 2.5 gm. of ferrous iron being distinctly inferior.

When it had appeared that in order to give a stable iron hematoxylin which did not overstain, a solution containing 15 gm. ferric ammonium sulphate (1.74 gm. ferric iron) and 15 gm. ferrous sulphate (3.01 gm. ferrous iron) in 100 cc. of distilled water, and 1 gm. of hematoxylin in 50 cc. of alcohol and 50 cc. of glycerine would be satisfactory, Experiment IV was set up to determine whether this solution could be diluted with water, or water, alcohol and glycerine without impairing its stability. The water, alcohol and glycerine diluent was made in the proportion 50:25:25. 5:5, 4:6, 2:8 and 1:9 dilutions were made with each diluent on August 24, 1938.

All dilutions deteriorated more rapidly than the stock solution, pure aqueous dilutions faster than the water, alcohol and glycerine dilutions and the higher dilutions fastest. While the stock solution had shown but little more intense staining in 30 than in 5 minutes, the aqueous dilutions showed marked overstaining when staining was prolonged. The water, alcohol and glycerine dilutions showed a less measure of the same change.

Since satisfactory myelin staining of previously chromated brain tissue may be obtained by overstaining with a neutral iron chloride or an iron alum hematoxylin, followed by differentiation with iron alum until gray and white substance can be distinguished, then treating with borax ferricyanide to develop the blue-black color of the myelin, it was thought that the overstaining with iron hematoxylin and the iron alum differentiation might be replaced by staining with an iron hematoxylin of the type under discussion which does not overstain. Solution III Q was used. This contains 15.12 gm. of ferric ammonium sulphate (1.75 gm. of ferric iron), 14.94 gm. of ferrous sulphate (3 gm. ferrous iron), 100 cc. of water; and 1 gm. of hematoxylin, 50 cc. of alcohol and 50 cc. of glycerine. Brain sections were stained 5, 10, 15 and 30 minutes, then treated 5 minutes with Weigert's borax ferricyanide. The staining effect was that of a plain iron hematoxylin stain, myelin not being colored at all.

Discussion and Summary

The foregoing experiments show that iron alum hematoxylin solutions containing 13 to 17.3 gm. of ferric ammonium sulphate (1.5 to 2 gm. ferric iron) in a 200 cc. quantity stain intensely in 5 minutes and do not overstain appreciably in 30 minutes. The addition of 15 to 20 gm. of ferrous sulphate (3 to 4 gm. ferrous iron) preserves the original blue-violet color of the fresh solution for at least 3 months and preserves satisfactory staining for at least 11½ months. Ferrous ammonium sulphate and ferrous sulphate may be used as sources of ferrous iron. Ferrous sulphate is to be preferred as it gives higher iron concentration for the same weight of salt and is more soluble.

It may be generally observed that solutions retaining their blue-violet color give satisfactory staining. Those showing purplish violet to purplish brown may also be quite usable, but those show-

ing a yellowish brown color in thin layers are generally inert.

Solutions containing 1.5 to 2 gm. of ferric iron in the 200 cc. unit quantity to 1 gm. of hematoxylin give adequate nuclear staining in 5 to 7 minutes and do not stain other tissue elements appreciably in 30 minutes. Solutions containing around 0.5 gm. of ferric iron (4.3 gm. ferric ammonium sulphate) stain promptly and intensely in 4 to 5 minutes and overstain in longer periods.

For preservation of the blue iron hematoxylin lake about twice as much ferrous iron as ferric is necessary, that is, quantities of ferrous sulphate or ferrous ammonium sulphate to give 3 to 4 gm. of ferrous iron (15 to 20 gm. ferrous sulphate).

A satisfactory solution which can be kept unchanged for several months is the following:

(A)	Ferric ammonium sulphate (violet crystals)	15 gm.
	Ferrous sulphate	15 gm.
	Distilled water	100 cc.
(B)	Hematoxylin	1 gm.
	Alcohol, 95%	50 cc.
	Glycerine, C P	50 cc.

Mix A and B in equal quantities.

While the foregoing type of solution is remarkably stable and effective, it is felt that greater stability can be attained as further studies now in progress indicate that such solutions may be attained.

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THE EFFECT OF YEAST ON THE INCIDENCE OF CIRRHOSIS PRODUCED BY LEAD ARSENATE *

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In a previous publication [1] were recorded the results of experiments in which copper arsenate, lead arsenate and sodium arsenate were employed to produce cirrhosis of the liver. It was found that these substances were effective as cirrhogenic agents for rabbits; furthermore, the incidence of cirrhosis could be reduced by certain diets.

When a diet of hay (alfalfa) and oats was fed the arsenates produced areas of necrosis in various parts of the liver lobules. These necrotic areas were sharply defined, the outlines of the necrotic cells were distinct and the cytoplasm did not stain with any of the dyes used. The necrotic cells did not contain any fat. With the doses of the arsenates employed the necrotic areas were situated most frequently adjacent to the portal region, often surrounded it, and in many instances extended from one portal region to another. Phagocytes collected about these necrotic liver cells. As the damaged liver cells disappeared, connective tissue was laid down and cirrhosis resulted.

When the diet of hay and oats was supplemented by the addition of carrots on one day and cabbage the next the incidence of cirrhosis was considerably reduced. With a diet of peeled white potatoes and white bread the incidence of cirrhosis was markedly reduced, even though the average amount of arsenic contained in the livers of these rabbits was greater than in those of the other two series.

It was then decided to determine whether other substances, added to the diet, might decrease the effect of arsenates on the liver. In the following experiments are reported the results of the addition of powdered brewer's yeast to the diet of rabbits given lead arsenate. This arsenate was chosen because it was so effective in producing cirrhosis and also because it is so widely used as an

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insecticide. The lead arsenate used was one of the commercial preparations for spraying and dusting and was from the same container as that used in the previous experiments. This had been analyzed and found to contain 98 per cent lead arsenate.

The experiment was carried out after the manner of those previously described. Only chinchilla rabbits were used; each weighed 1250 to 1500 gm. at the beginning of the experiment. Under ether anesthesia a piece of liver was removed to serve as a control for any alterations that might subsequently occur. The animals were allowed to recuperate for at least 2 weeks. They were given a diet of hay (alfalfa) and oats throughout the entire period of the experiment; this diet will hereafter be referred to as the standard diet. The arsenate was thoroughly mixed with some starch and was sprinkled on a small quantity of oats; after these had been eaten the standard diet was allowed in liberal quantity. The brewer's yeast was administered in the same manner as the arsenate and when given with the arsenate it was mixed with the same oats.

The biopsy specimens of liver were fixed in a solution of formaldehyde U.S.P. (1:10). The autopsy material was fixed in a formaldehyde solution (1:10), and in Zenker's solution without the addition of either acetic acid or formalin. Sections were stained with hematoxylin and eosin and with the stain for the differentiation of hemosiderin and hemofuscin (Mallory, Parker and Nye [2]). Frozen sections of the liver were stained with scharlach R. In addition, specimens of many of the livers were fixed in absolute and in 95 per cent alcohol, and sections from them were stained for glycogen according to Best's method. Arsenic determinations were made by the Gutzeit method. The yeast was analyzed for arsenic but only a trace was found.

Standard Diet

Four rabbits were fed the standard diet without the addition of lead arsenate or yeast. They were kept on the diet for 265 days, when they were sacrificed. The rabbits increased in size steadily and were very fat. The livers from this group of rabbits were free from cirrhosis. In 1 rabbit the pigment in the liver cells was slightly increased, in another the amount of pigment was not changed, and in 2 it was slightly decreased. There were no phago-

cytes in any of the livers. Arsenic was not present in any of the livers or kidneys (Table II).

Standard Diet and Yeast

There were 5 rabbits in this series. Each was given 3 gm. of powdered yeast daily. The animals were sacrificed at the end of 384 days. At this time they were very large and fat, their fur unusually thick and long.

There were no hepatic lesions in this group nor were there any phagocytes. In 2 of the rabbits the hepatic pigment was not increased, in 2 it was slightly increased, while in the other there was a moderate increase of pigment. There was no arsenic in the livers or kidneys (Table II).

Standard Diet and Lead Arsenate

Five rabbits were given daily doses of 7.2 mg. of lead arsenate (1.4 mg. of arsenic). The 1st animal of this group died on the 8th day and none lived longer than 26 days. In no instance could any gross abnormality of the liver be detected. In 2 of the rabbits (Nos. 410 and 412) a few areas of hepatic necrosis were found; in the other 3 animals areas of necrosis were numerous. These areas were, most often, adjacent to the portal regions, though they were found also in other parts of the lobule. Frequently the areas of necrosis extended from one portal region to another. They were sharply defined from the surrounding liver cells. The necrotic hepatic cells had distinct outlines, were swollen, somewhat rigid in appearance and the cytoplasm rarely stained with eosin. No nuclei could be seen in many of the necrotic cells; in other cells the nuclei were fragmented or pyknotic. These necrotic liver cells did not contain fat. The endothelium of the sinusoids in the areas of necrosis appeared unharmed. Large phagocytes were frequently collected about the necrotic liver cells. In some instances these phagocytes were numerous and many of them were multinucleated. At times similar phagocytes were present in the portal areas. There was as yet little evidence of the laying down of connective tissue in the areas of necrosis.

In the 2 rabbits (Nos. 412 and 417) that survived for 26 days the connective tissue of the portal areas was increased, but not strikingly so, and did not pass to any of the neighboring portal

areas. This is designated as early cirrhosis. The bile ducts were beginning to proliferate.

The pigment in the liver cells was not increased in 2 of the animals; 1 of these died on the 8th day, the other on the 15th day. There was a slight increase of pigment in the liver cells of the animal that died on the 14th day; in the other 2 rabbits the increase of the hepatic pigment was moderate. The phagocytes were often filled with pigment similar to that seen in the liver cells.

Another series of 7 rabbits was fed the standard diet and given lead arsenate. The daily dose of the arsenate for the first 52 days was 2.4 mg. (arsenic 0.4 mg.); the amount of the arsenate was then increased to 4.8 mg. (1 mg. arsenic). Two rabbits died after having received the increased doses of arsenate for 40 days; these animals had thus received arsenate over a period of 92 days. The other 5 rabbits were continued on the arsenate dosage of 4.8 mg. for 118 days. The amount of arsenate was then increased to 7.2 mg. (1.4 mg. arsenic); after 8 days on this dosage the animals were sacrificed. These 5 animals received the arsenate for 178 days.

Only 1 rabbit (No. 422) had a grossly normal appearing liver; of the other 6, the livers of 4 had an irregular capsular surface and on section the cut surface was browner than normal and appeared moist. The livers of the 2 remaining rabbits, while having a smooth external surface, had a moist edematous cut surface, and in 1 of these the lobulation was irregular.

In none of these 7 rabbits were areas of hepatic necrosis found nor were any individual liver cells necrotic. However, in 6 rabbits connective tissue in the portal areas was increased. In 4 animals the connective tissue did not pass to any of the adjacent portal areas; in 2, bands of connective tissue extended to neighboring portal areas. There was no irregularity of lobular outline in any of these livers. Following the classification used in the previous report to designate the degree of cirrhosis, the 4 examples in which the connective tissue was limited to the portal area are classified as early, the other 2 as moderate. The 1 rabbit in the group that had a liver free from cirrhosis was No. 422.

Phagocytes were found in each of the livers, although they were not so numerous as in the other rabbits with areas of hepatic

necrosis. The phagocytes were present in the portal areas and sinusoids; many of them were multinucleated.

Hepatic pigment was not increased in the liver of 1 rabbit (No. 425); in the others the pigment was increased, although not markedly so. It was present in hepatic cells, Kupffer cells and phagocytes. There was proliferation of bile ducts in 4 of the rabbits (Nos. 416, 423, 425 and 426). The quantities of arsenic found in the livers and kidneys are shown in Table I.

Standard Diet, Lead Arsenate and Yeast

Thirteen rabbits were fed the standard diet and to each of them was given daily 7.2 mg. of lead arsenate (1.4 mg. of arsenic) and 3 gm. of yeast. In Table I are shown the various intervals the rabbits survived. It will be noted the first death occurred on the 58th day. Rabbits Nos. 374 and 379 were sacrificed on the 384th day to terminate the experiment.

The external surface of the liver of each of these rabbits was normal; in only 3 instances (rabbits Nos. 370, 371 and 407) could any increase of connective tissue in the portal areas be detected when the liver was sectioned. Necrotic areas such as have been described in the animals given similar daily doses of the arsenate were found in only 3 of the livers. These areas were very few in number and were situated in various parts of the lobule. Necrosis of individual liver cells was found only in the liver of 1 rabbit (No. 374). These necrotic cells were situated near the portal regions and their appearance was similar to that of the cells in areas of necrosis. The connective tissue of the portal area was somewhat increased in 3 of the livers but did not, however, pass to any of the adjacent lobules and the cirrhosis is therefore designated as early. In 3 other instances there was a greater increase of connective tissue in the portal areas with strands of connective tissue at times passing to nearby portal areas (moderate cirrhosis). Proliferation of bile ducts was found in 5 instances (rabbits Nos. 370, 371, 375, 380 and 407), but the proliferation of the ducts was not a striking feature. It will be noted that this duct proliferation occurred only in those livers that were cirrhotic.

Phagocytes were not found in 4 of the livers (rabbits Nos. 366, 372, 375 and 378); a few were found in 3 (Nos. 369, 373 and 379), and they were more numerous in the other livers, especially

those in which cirrhosis was present. The phagocytes were situated near the necrotic areas, in portal areas and in the sinusoids, and were at times multinucleated.

Pigment was increased in the liver cells of 11 of the animals;

TABLE I

Résumé of Hepatic Lesions in Rabbits Given Lead Arsenate or Lead Arsenate and Yeast

Rabbit No.	Lead arsenate, daily dose	Days	Necrotic areas in liver	Cirrhosis	Arsenic	
					Liver	Kidney
			Lead Arsenate			
	mg.				*mg. per 100 gm. dry wt.*	
413	7.2	8	Numerous	None	0.500	
409	7.2	14	"	"	0.500	0.320
410	7.2	15	Few	"	0.500	0.640
412	7.2	26	"	Early	2.093	1.230
417	7.2	26	Numerous	"	2.350	4.050
425	a	92	None	Moderate	0.667	0.400
426	a	92	"	"	0.667	0.400
415	b	178	"	Early	0.500	0.500
416	b	178	"	"	0.667	0.532
419	b	178	"	"	Traces	Traces
422	b	178	"	None	0.500	0.400
423	b	178	"	Early	0.500	0.250
			Lead Arsenate and Yeast			
372	7.2	58	None	None	....	....
366	7.2	62	"	"	....	....
378	7.2	62	"	"	....	....
370	7.2	98	Few	Moderate	1.000	....
377	7.2	137	None	Early	0.170	....
371	7.2	154	Few	Moderate	1.700	1.320
407	7.2	172	None	"	1.000	0.440
369	7.2	174	"	None	1.330	0.600
373	7.2	178	"	"	1.000	0.908
380	7.2	181	Few	Early	0.660	0.276
375	7.2	310	None	"	0.165	0.200
374	7.2	384	"	None	0.330	0.332
379	7.2	384	"	"	0.267	0.224

a = 2.4 mg. lead arsenate (0.4 mg. arsenic) daily 52 days.
4.8 mg. lead arsenate (1.0 mg. arsenic) daily 40 days.

b = 2.4 mg. lead arsenate (0.4 mg. arsenic) daily 52 days.
4.8 mg. lead arsenate (1.0 mg. arsenic) daily 118 days.
7.2 mg. lead arsenate (1.4 mg. arsenic) daily 8 days.

it was also seen frequently in Kupffer cells and usually in the phagocytes.

The arsenic determinations of the livers and kidneys are shown in Table I.

Pigment

Pigment was present in biopsy specimens of the liver from each of the rabbits. It was finely granular, yellow and refractive. The amounts and distribution varied in different animals. The pigment did not contain iron.

The pigment found in the livers at autopsy had the same appearance as that in the biopsy specimens although the granules were frequently larger. It did not give a reaction for iron but stained red with scharlach R. The greatest increases of pigment were in those animals that received the arsenate.

Glycogen Determinations *

Glycogen analyses were made on the livers of the 4 diet control rabbits, the 5 rabbits given the standard diet and yeast, 5 of the rabbits given lead arsenate, and 2 of those that received lead arsenate and yeast. The results are shown in Table II, together with the amounts of arsenic found in the livers and kidneys. The glycogen was determined by the method of Good, Kramer and Somogyi.[3] The animals were killed in the forenoon. Care was exercised to obtain the specimens of liver for glycogen analysis as quickly as possible after the animals had been killed. It will be noted from a study of the table that there is great variation in the amounts of glycogen found in different animals subjected to the same experimental procedure, as well as in those of different groups. The greatest average amount was found in the group of rabbits that received yeast; the next largest average amount was in the livers of the animals that received arsenate. The average amount of glycogen found in the livers of the arsenate-yeast rabbits was comparable to that of the diet control group. There was no correlation between the glycogen and arsenic content of the livers.

Sections of liver were stained by Best's method and the distribution of glycogen within the liver lobule was studied. Among the diet control group, with one exception, glycogen was found in the liver cells throughout the lobule, although it was most abundant in those cells surrounding the efferent veins. The one exception was rabbit No. 343; in this rabbit the glycogen could be demon-

* We are greatly indebted to Dr. Louis B. Dotti for assistance with the glycogen determinations.

strated only in widely separated liver cells situated in various parts of the lobule.

The most regular distribution and even concentration of glyco-

TABLE II

Glycogen Content of the Liver

Rabbit No.	Days	Glycogen content of liver	Arsenic		Cirrhosis
			Liver	Kidney	
		Diet Controls			
		%	*mg. per 100 gm. dry wt.*		
343	265	1.000	0	0	None
339	"	1.750	0	0	"
341	"	1.845	0	0	"
354	"	2.135	0	0	"
		1.682 Average			
		Yeast Controls[1]			
405	384	1.860	0	0	"
406	"	2.510	0	0	"
401	"	2.810	0	0	"
404	"	3.260	0	0	"
402	"	3.540	0	0	"
		2.796 Average			
		Lead Arsenate[2]			
415	178	1.570	0.500	0.500	Early
423	"	1.710	0.500	0.250	"
422	"	2.040	0.500	0.400	None
416	"	2.330	0.667	0.532	Early
419	"	2.920	Traces	Traces	"
		2.114 Average			
		Lead Arsenate and Yeast[3]			
374	384	1.000	0.330	0.332	None
379	"	2.000	0.267	0.224	"
		1.500 Average			

[1] 3 gm. powdered brewer's yeast daily.

[2] 2.4 mg. lead arsenate (0.4 mg. arsenic) daily 52 days.
4.8 mg. lead arsenate (1.0 mg. arsenic) daily 118 days.
7.2 mg. lead arsenate (1.4 mg. arsenic) daily 8 days.

[3] 7.2 mg. lead arsenate (1.4 mg. arsenic) daily 384 days.
3 gm. powdered brewer's yeast daily 384 days.

gen were found in the livers of the rabbits that received yeast. In this group of animals each liver cell in the lobule appeared to hold an equal amount of glycogen.

A definite and uniform irregularity of glycogen deposition was

found in 3 of the rabbits that received only the arsenate. About most of the portal areas in the sections of liver from each of these animals (rabbits Nos. 415, 422 and 423) was a zone where the liver cells had no demonstrable glycogen. These zones were several cells wide and frequently extended to the adjacent portal areas. These glycogen-free cells in the hematoxylin-eosin preparations had well preserved, normal appearing nuclei and were of the same size as the hepatic cells about the efferent veins. The only difference that could be detected was that the cytoplasm of the glycogen-free cells was less granular than that of the cells filled with glycogen; this difference, however, was not striking. The glycogen in the livers of the other 2 animals of this group was uniformly distributed throughout the lobule.

In 1 of the 2 rabbits (No. 374) that was given arsenate and yeast the glycogen was deposited chiefly in the liver cells about the efferent veins and portal areas; in the midzonal portion of the lobules glycogen was much less abundant and was in widely separated single cells. In this animal an occasional isolated cell, immediately adjacent to the portal areas, had sharply defined borders and clear cytoplasm. These cells resembled those of the necrotic area produced by arsenic in their staining with hematoxylin and eosin. In the section stained by Best's method similar cells were observed to be more uniformly filled with glycogen than were the neighboring liver cells. This suggests the possibility that the damaged hepatic cells in the necrotic areas are filled with glycogen. The glycogen in the liver of the other animal of this group was found in the hepatic cells throughout the lobule but the deposition was greatest in those cells about the efferent vein.

Discussion

There are obvious differences in the groups of rabbits that received the arsenate and those given the arsenate and yeast. Of the 5 rabbits given 7.2 mg. of the arsenate daily, none lived longer than 26 days and of these 2 had early cirrhosis. The characteristic areas of necrosis were found in each of the livers, being very numerous in 3 rabbits. In the second control group given smaller amounts of the arsenate, necrosis was not found although cirrhosis was present in 6 of these 7 rabbits. It would seem, therefore, that the arsenate in small doses will produce a slowly developing

cirrhosis without the sharply defined areas of necrosis. Among the group of 13 rabbits given 7.2 mg. of the arsenate and 3 gm. of yeast daily, the 1st animal to succumb died on the 58th day. This is more than twice the length of time the last survivors of the corresponding control group lived. The addition of yeast had a distinct effect in prolonging the lives of the rabbits. Another difference noted is in the frequency of the characteristic areas of hepatic necrosis. These were found in only 3 of the 13 rabbits given yeast and arsenate. Furthermore, these areas were few in number, while in the 5 animals given a similar amount of the arsenate without yeast they were very numerous in 3.

When the incidence of cirrhosis in the group of 13 rabbits given the arsenate and yeast is compared with that in the group receiving the smaller doses of the arsenate, a difference is apparent. In the arsenate and yeast series 6 of the 13 animals had cirrhosis, an incidence of approximately 46 per cent; in the group given only the arsenate, cirrhosis was found in 6 of the 7 rabbits, approximately 85 per cent.

The amounts of glycogen in the livers is so variable no very definite conclusion can be drawn. The highest average value was found in the livers of the animals given yeast daily as a supplement to the standard diet. That the increased glycogen content of these livers may be attributed to the yeast is indicated from the experiments of Bickel and Nigmann,[4] Bickel and Collazo,[5] and Labbé, Nepveux and Gringoire.[6] But this explanation cannot be offered for the somewhat higher average of glycogen content in the liver found in the rabbits that received arsenate only, as compared with those of the diet controls.

The lower incidence of cirrhosis among the animals given the arsenate and yeast is in accord with results of the recently reported experiments of Nakahara, Fujiwara and Mori.[7] These investigators using dimethyl-amino-azo-benzol to produce liver cell carcinoma in rats found that the incidence of cirrhosis and carcinoma was reduced when the diet of polished rice was supplemented by yeast.

What the protective substance in the yeast may be has not yet been determined.

Conclusions

The incidence of hepatic cirrhosis in rabbits produced by the ingestion of lead arsenate is reduced when powdered brewer's yeast is added to the diet.

There is no apparent relation between the amount of hepatic glycogen and the quantity of arsenic in the liver, nor is there any obvious connection between the glycogen content of the liver and the incidence of cirrhosis.

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PRIMARY CHORIONEPITHELIOMA OF THE URINARY BLADDER IN A MALE *

Report of a Case

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Truly ectopic chorionepithelioma in the male is rare. Up to 1933 Heaney [1] found 131 reported cases of chorionepithelioma in the male and with a few exceptions the primary tumor was in the testicle. Only 6 cases of ectopic chorionepithelioma in which the testes were carefully examined and definitely excluded as the primary site have been reported to date. The following case makes the 7th reported to date.

Report of Case

Clinical History: J. J., a white male, aged 70 years, formerly a hospital orderly, was admitted to the urological service of The Mount Sinai Hospital Dec. 1, 1938, with the complaint of hematuria, dysuria and frequency. Four months before admission he noticed discomfort on urination and a sensation of "gravel" in the urethra. Two months later he began to have frequency, urinating every 3 to 5 minutes, and noticed that the urine was blood tinged. At times blood clots would form and prevent urination, the latter being completed only after the passage of the clots, some of which measured 2.5 to 6.5 cm. in length. During this time there was severe pain on urination, which continued throughout micturition. Five weeks before admission to the hospital the patient felt as though the bladder were distended. On passing a catheter it was found that the presence of blood clots had prevented urination for almost 8 hours. A large amount of urine was obtained which was bloody and full of clots. For over a month the patient had also noticed occasional streaks of blood in the sputum. Five days before admission to the hospital marked dyspnea and cyanosis developed.

Physical Examination: This revealed a dyspneic and cyanotic elderly individual who manifested marked discomfort. No râles were heard in the chest. Examination of the heart was entirely negative. The blood pressure was 120 mm. Hg. systolic and 50 diastolic. The nipples were sensitive to the touch. There was general tenderness over the entire abdomen, particularly in the right upper quadrant, the epigastrium and the right lumbar region. The liver was easily palpable in the epigastric and right hypochondriac regions and was painful to the touch. The prostate was found to be moderately enlarged.

Laboratory Data: The hemoglobin (Sahli) was 50 per cent. The urine

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was grossly tinged with blood, was alkaline in reaction and had a specific gravity of 1.020. The test for albumen was 1 plus. Culture of the urine revealed *Staphylococcus albus A* and enterococcus. A cystogram was done but was unsatisfactory because of an inadequate amount of opaque material within the bladder. The blood urea on admission was 28 mg. per cent. The venous pressure was 11 cm. with a rise to 14.5 cm. on pressure in the right upper quadrant of the abdomen. The saccharine circulation time was 17 seconds. An electrocardiogram revealed regular sinus rhythm with frequent auricular premature contractions. There was left ventricular preponderance.

Course of Illness: The temperature on admission was 101.4° F. Throughout the patient's stay in the hospital the temperature ranged between 101 and 102° F. Three days after admission it was noted that the patient showed evidence of heart failure on the right side. The veins of the neck were distended and the liver was enlarged to three finger breadths below the costal margin. There was pitting edema over the sacrum and legs. Bilateral basal râles were present in the chest. Digitalis and diuretics were prescribed. At this time an indwelling catheter was left in the bladder. One week after admission to the hospital the blood urea rose to 61 mg. per cent. A small transfusion was given but the patient's general condition became steadily worse. He passed into a delirium with Cheyne-Stokes respiration and died 12 days after admission. A cystoscopy to determine the cause of the hematuria was not attempted because of the precarious condition of the patient.

Postmortem Examination

The autopsy was performed 1 hour and 15 minutes after death. The essential changes noted were as follows:

The sclerae showed a slight icteric tinge. The breasts were not enlarged and contained no palpable nodules. The distribution of hair was normal. A small dermoid cyst 1.5 cm. in diameter was present within the skin of the anterior abdominal wall in the midline in the epigastrium. The lower border of the liver was situated 10 cm. below the costal margin in the right midclavicular line. The bladder was of approximately average size and was rigid and firm on its posterior and right lateral aspects, being densely adherent to the right side of the pelvic wall. When the bladder was opened it was found to contain fragments of pinkish gray, friable material mixed with bloody fluid. A tumor mass measuring 7 by 8.5 cm. (Fig. 1) involved the right half of the trigone and the entire right and the greater portion of the posterior walls which were greatly thickened and rigid, varying in width from 2 cm. to approximately 5 cm. in the trigonal region. The mucosal aspect of the tumor presented a fungating, pinkish gray sloughing area which was raised above and distinctly demarcated from the more normal, smooth grayish pink mucosa. A

small, firm, grayish yellow ulcerated tumor nodule 3 mm. in diameter projected above the surrounding mucosa in the left fundal region. Section through the tumor mass presented a mottled, dark grayish red appearance. Other areas were distinctly hemorrhagic. When viewed from the cul-de-sac aspect an extensive, mottled, irregular hemorrhagic area was seen beneath the smooth and glistening peritoneum covering the posterior wall of the bladder. This area was moderately firm and slightly nodular to the touch. The right ureteral orifice could not be found nor could it be probed from above by introduction of a catheter into the ureter. The left ureteral orifice was patent but was slightly encroached upon by the hemorrhagic tumor mass which terminated in juxtaposition to it. The prostate was not enlarged and not involved by the tumor. The seminal vesicles were surrounded by tumor tissue. A small bilateral hydrocele was present. The testes were similar and normal in size and shape. Serial sections 1 to 2 mm. in thickness through both testes and epididymes failed to reveal any abnormal features. The spermatic and lower pelvic veins contained phleboliths. Both ureters were dilated, the right being larger and containing about 50 cc. of bloody, dark red urine. The right kidney was smaller than the left and showed a moderate degree of hydronephrosis with atrophy of the parenchyma. Both kidneys contained deeply hemorrhagic tumor nodules of varying size. A bilateral adrenonephric symphysis was present. The liver was markedly enlarged and weighed 3220 gm. Its surface was studded with numerous firm tumor nodules varying from 1 to 6 cm. in diameter. Some of these nodules had umbilicated centers, others were hemorrhagic in appearance, and a few were yellowish white with dark red flecks. Cut surface of the liver revealed replacement of a greater portion of the liver parenchyma by well demarcated tumor masses, most of which had a mottled, dark red honeycombed appearance. Most of the smaller nodules, however, were a pale yellow color with dark red streaks coursing through them. The lungs were voluminous and weighed 2860 gm. Rounded, nodular, bulging tumor masses varying in diameter from 0.5 to 6 cm. were seen on all surfaces of the lungs. A few nodules were subpleural, had umbilicated centers, and were a mottled dark red color. Deeply embedded masses were also present which produced distinct bulges on the surface of the lungs. These

tumor masses were covered by normal intervening parenchyma. On cut section (Fig. 2) each lung appeared riddled with distinctly demarcated tumor nodules of varying size which projected above the cut surface. The larger tumor nodules were friable and dark red, and appeared indistinctly honeycombed. The centers of the nodules were soft in consistence. The smaller tumor nodules were for the most part a pale yellow color with reddish areas. Between the nodules were areas of bronchopneumonia. Considerable emphysema was present. In the gross, similar metastatic tumor nodules were seen in the ribs, sternum and vertebrae, epicardium of the heart, spleen, adrenals, pancreas, omentum, mesentery, small intestine and the tracheobronchial, para-aortic and cervical lymph nodes. The pituitary was normal in size and on sagittal section revealed small flecks of soft whitish tissue about 1 mm. in diameter which appeared to be located at the junction of the anterior and posterior lobes. A single small hemorrhagic tumor nodule was found in the right occipital lobe of the brain.

Biological Examination

Urine: The *rat test* for gonadotropic hormone gave a positive reaction with 0.1 cc. of urine, which is equivalent to 10,000 rat units per liter. This corresponds to the pregnancy titer.

The *rabbit test* disclosed more than 1000 rabbit units of gonadotropic hormone in each liter of urine, which is a strong reaction, as in the test for pregnancy 20 cc. of urine are required for a positive reaction. Tests for estrogenic hormone were negative.

Tumor Tissue (*Lung Metastases*): Extracts of tumor tissue from the lungs failed to show either gonadotropic or estrogenic activity.

Microscopic Examination

Sections through the bladder in the region of the tumor mass showed the marked thickening to be due to infiltrating sheets of tumor cells which penetrated all coats of the bladder and extended posteriorly to surround the seminal vesicles. The superficial layers were necrotic and hemorrhagic. Similar areas were present in the deeper layers. The more intact portions were composed of cells resembling the Langhans' cells of normal placental tissue (Fig. 3). They were large polyhedral cells with a basophilic

vacuolated cytoplasm and large vesicular nuclei. Occasional mitoses were present. Scattered among the Langhans' cells were large cells with irregular outlines and an acidophilic cytoplasm containing multiple hyperchromatic nuclei which conformed in appearance with that of the syncytial cells of the placenta (Fig. 4). No cilia were found on their borders. Small nests of Langhans' cells were seen within submucosal blood vessels of the bladder and in vessels about the seminal vesicles. The nodules of tumor tissue in the other organs were similar histologically with the degree of necrosis and hemorrhage varying in the individual nodules. Those in the lungs and liver compressed the surrounding normal parenchyma. Nowhere were vessels seen within the tumor masses, the blood being free in spaces between sheets of tumor cells. Moderate amounts of glycogen were present within the Langhans' cells. Microscopic metastases were present in the thyroid and the posterior lobe of the hypophysis. The latter organ contained scattered groups of large, irregularly shaped cells suggestive of "pregnancy cells." The testes were examined by serial block section and showed no microscopic evidence of chorionepithelioma. Spermatogenesis was moderately active but scattered atrophic and fibrotic tubules were also seen. Nodular areas of interstitial cell hyperplasia were present. The parathyroids were normal. Areas of chronic and acute pneumonitis were present in the lungs.

Discussion

Reports in the literature of proved primary ectopic chorionepithelioma in the male in which testicular examinations have been thoroughly and completely carried out are few in number. Kantrowitz,[2] in addition to reporting an undoubted case primary in a teratoma of the mediastinum, reviewed the literature up to 1934 and found only 4 cases which fulfilled the criteria proposed by Prym [3] (thorough examination of the genital tract, particularly the testes) — those of Miller and Browne,[4] Krassnianskaya,[5] Arendt,[6] and Heaney.[1] Gerber [7] added 1 more case, making a total of 6 acceptable ectopic cases in the male. All tumors were situated either in the mediastinum, the lung, or retroperitoneally. Three were composed purely of chorionepitheliomatous elements, while the remaining three had a demonstrable origin in a teratoma.

Search of the literature for primary chorionepithelioma of the

bladder yielded 5 cases. Two were in males and 3 in females. Critical analysis of these cases makes it doubtful if all can be accepted as primary tumors of the bladder, or even as chorionepitheliomas.

Djewitzki's [8] case was in a 75 year old virgin female, who had had vaginal bleeding for 2 years. Dysuria with reddish urine had also been present. Dilatation and curettage of the uterus revealed a hyperplastic endometrium. At autopsy multiple fibromyomas were found in the uterus. The ovaries were small and atrophic. The tubes and vagina were negative. Situated on the posterior wall of the bladder was an elevated, dark red, centrally ulcerated tumor measuring 5 cm. in diameter. Metastases were present in the lungs, bronchial lymph nodes, spleen and sigmoid. Microscopically both the tumor of the bladder and the metastases showed the typical appearance of Marchand's [9] chorionepithelioma. The examination seems to have been quite thorough and this must be accepted as the 1st reported case of chorionepithelioma of the bladder.

Venulet [10] reported the case of a male, aged 30 years, in whom autopsy revealed a polypoid papillary tumor 0.5 cm. in diameter at the right ureteral orifice. Metastases were present in the lungs, mesentery, retroperitoneal lymph nodes and the pelvic space posterior to the bladder. Ascites and hydrothorax were present. The testes were said to be negative but the thoroughness of their examination cannot be ascertained. Microscopically the tumor showed both Langhans' and syncytial cell groups, but the author did not believe he was dealing with a malignant chorionepithelioma because of lack of hemorrhage in the tumors. He considered it to be one of carcinoma with abnormal cell formation and compared it with Risel's [11] and Davidsohn's [12] cases of carcinoma of the stomach with chorionepithelioma-like metastases. The author's illustrations are of no assistance concerning the origin of the tumor. The probability of incomplete examination of the testes must be borne in mind and this case put on the "doubtful" list.

Blecher and Martius' case [13] was that of a 21 year old male in whom cystoscopy revealed a small tumor on the anterior and superior walls of the right side of the bladder. At operation it was found to be intramural, well circumscribed and completely covered

by mucous membrane. On section it was composed of a number of tumor nodules which showed areas of hemorrhage. Microscopically the tumor failed to show the typical combination of Langhans' and syncytial cells, but instead areas of spindle shaped cells and others with dense medullary columns. Still other areas showed a syncytial arrangement with loss of cell boundaries, giant cells, and also vacuolated cells. The tumor invaded the lymphatics. The testes appeared normal. The patient was free of recurrence 1 year after operation. The authors could not arrive at any definite diagnosis but referred to the similarity to chorionepithelioma on the one hand and "endothelioma" on the other. The author's description and illustrations rather suggest a myosarcoma of the bladder. It certainly cannot be accepted unreservedly as a chorionepithelioma, particularly since the patient was still alive and well 1 year after simple resection of the tumor. The lack of an autopsy follow-up affects the value of this case.

Charkviani [14] reported a case in a 32 year old female who had come to the clinic complaining of vaginal bleeding. A hydatid mole was discovered and the uterus was removed. Subsequently the patient returned and a necrotic tumor of the bladder was found. A tumor nodule was also found in the vagina and was diagnosed as chorionepithelioma on biopsy. X-radiation was applied and the patient improved and was well a year later. Obviously in the presence of the hydatid mole in the uterus the diagnosis of the tumor of the bladder as a primary chorionepithelioma is not justifiable. Furthermore, no report is given of a biopsy of the tumor of the bladder.

Pazourek [15] published a case which apparently was a primary tumor of the bladder, but here again unreserved acceptance is not forthcoming. The patient was a 26 year old married female. A tumor situated on the left bladder wall just above the ureteral orifice on that side was removed by partial resection of the bladder. The author claimed the microscopic appearance was typical of chorionepithelioma but his illustrations do not lend conviction to this conclusion. A curettage was performed soon after resection of the bladder and showed only atrophic endometrium. Pazourek then concluded he was dealing with a primary chorionepithelioma of the bladder which had developed in a "kyste hydatique." The patient was perfectly well 7 months after operation.

From the analysis of the cases reviewed above it is obvious that we are left with only 1 acceptable case of primary chorionepithelioma of the bladder, the 4 others reported in the literature being either too vague in their descriptions and illustrations or obviously not even primary tumors of the bladder. It is important to note that none of these cases had hormonal studies.

Reports of hormone studies in cases of chorionepithelioma in the male are few. Such studies were first carried out by Heidrich, Fels and Mathias in 1930.[16] In our case the rabbit test for gonadotropic hormone in the urine was strongly positive, the test being positive with 1 cc. of urine. In normal pregnancy 20 cc. of urine are customarily necessary to obtain a positive reaction. The rat test gave a positive reaction with 0.1 cc. of urine which is equivalent to 10,000 rat units per liter, which in turn corresponds to the titer usually found in pregnancy. Owen and Cutler [17] consider any reaction from 10,000 to 125,000 m.u. or more per liter in the male as indicative of chorionepithelioma. Belt [18] says that the hormone output is in proportion to the extent and growth of the metastases and that in cases where the metastases are predominantly hemorrhagic in character few hormone active and stimulating cells may be left. The failure to extract gonadotropic or estrogenic substance from the pulmonary metastases in our case may possibly be explained by the partial hemorrhagic necrosis of the tumor nodules. No estrogenic hormone was found in the urine.

Although the morphology of the tumor in our case is characteristically that of a chorionepithelioma, the demonstration of biological activity, as evidenced by the excretion of large amounts of gonadotropic hormone in the urine, leaves no doubt as to its true nature.

Chorionepithelioma arising either retroperitoneally or within the mediastinum is said to have its origin either in germinal rests [7, 19, 20] (*i.e.* the plica urogenitale), or in malignant transformations, as well as in unilateral development of teratomas.[21, 22, 23] Accordingly, its occurrence retroperitoneally or in the mediastinum can be well understood, but its origin in the bladder remains unexplained. For teratomas arising in the bladder the theory has been suggested that they are derived from "dysontogenetic rests of the dorsal mesodermal segments which are carried down to the bladder anlage by the caudally growing

wolffian duct." [24] Therefore a chorionepithelioma arising in a teratoma of the bladder would have an acceptable mode of development. But when a chorionepithelioma containing no teratomatous elements arises in this region the explanation of its origin is not so simple unless one assumes its origin to be from a teratoma which has developed unilaterally.

Although teratomatous elements were not found in the sections of the primary tumor in the bladder in the case reported here, it cannot be stated definitely that the tumor did not have its origin in a teratoma because, as Kantrowitz [2] has pointed out, the remnants of such elements may be so few and so well localized as to defy their discovery except by the examination of serial sections through the entire tumor. Recalling the size of the neoplasm in this case such an enterprise would be impossible from a practical point of view. In the cases reported in the literature the metastases from ectopic chorionepitheliomas arising in teratomas almost invariably showed no teratomatous elements — only chorionepithelioma. The metastases in our case were of a similar nature.

Although not as striking as in the case of Kantrowitz,[2] nodular hyperplasia of the interstitial cells of the testes was quite evident. A similar finding has been recorded by Arendt [6] and by Heaney.[1] Its significance is unknown.

That certain specific changes occur in the pituitary during pregnancy are well known. In addition to the usual three types of cells in the anterior lobe a cell is present which has certain of the characteristics of both the eosinophil and the basophil, but which differs from them in the size of the cell, the position of the nucleus and the staining qualities. This is the so-called "pregnancy cell" which has its origin in the chromophobe cells of the anterior lobe. The chromophobe cells are transformed into large cells, the cytoplasm of which is filled with fine dust-like granules which stain pink with eosin, and the nuclei are irregular and vesicular. The size of the granules may be contrasted with the much coarser granules found within the cytoplasm of the eosinophils. Actually neither the normally predominant eosinophils nor the basophils vary in number during pregnancy, but an apparent alteration in proportions is brought about by the great increase in modified chromophobe or pregnancy cells. It is noteworthy that changes

similar to those found in pregnancy are also found frequently in carcinoma, particularly the cases with extensive metastases to the liver.[25]

The appearance of the pituitary in pregnancy, as noted above, has also been described in cases of chorionepithelioma in the male.[26–36] However, the number of reports containing descriptions of the pituitary are relatively few and among these are diverse findings. While the majority of authors describe various degrees of "pregnancy change," some describe a less distinctive change and one, in fact, reports no change at all. Stöckl's case [31] goes to the other extreme in that he finds a marked increase and predominance of basophilic cells. In our case the proportions of basophils to eosinophils appeared to be within normal limits. However, scattered throughout the anterior lobe were a moderate number of large, irregularly shaped cells which were distinctly reminiscent of "pregnancy cells." Their cytoplasm was filled with fine granules which showed an affinity for the acid stains. Many of the basophils and eosinophils showed vacuole formation in the cytoplasm.

Summary and Conclusions

A primary chorionepithelioma of the urinary bladder in a 70 year old male is reported, the 2nd case to appear in the literature.

The testes, vas deferens, seminal vesicles and prostate showed no tumor involvement. Sections from 1 to 2 mm. in thickness were made throughout the entire thickness of both testes and microscopic slides were made from each block. No tumor nodules were found. Slight tubular atrophy and nodular interstitial cell hyperplasia were present.

Sections through the primary tumor in the bladder failed to reveal any teratomatous elements. However, the possibility of the unilateral development of this tumor from a teratoma cannot be disproved.

The incomplete "pregnancy" reaction in the pituitary as well as metastatic involvement of the same organ is discussed.

Both the rabbit and the rat tests for gonadotropic hormone were significantly positive in the urine. Tests for estrogenic hormone were negative. Extracts of tumor tissue from the lungs failed to show either gonadotropic or estrogenic activity.

The origin of the tumor in dysontogenetic rests of the dorsal mesodermal segments is suggested.

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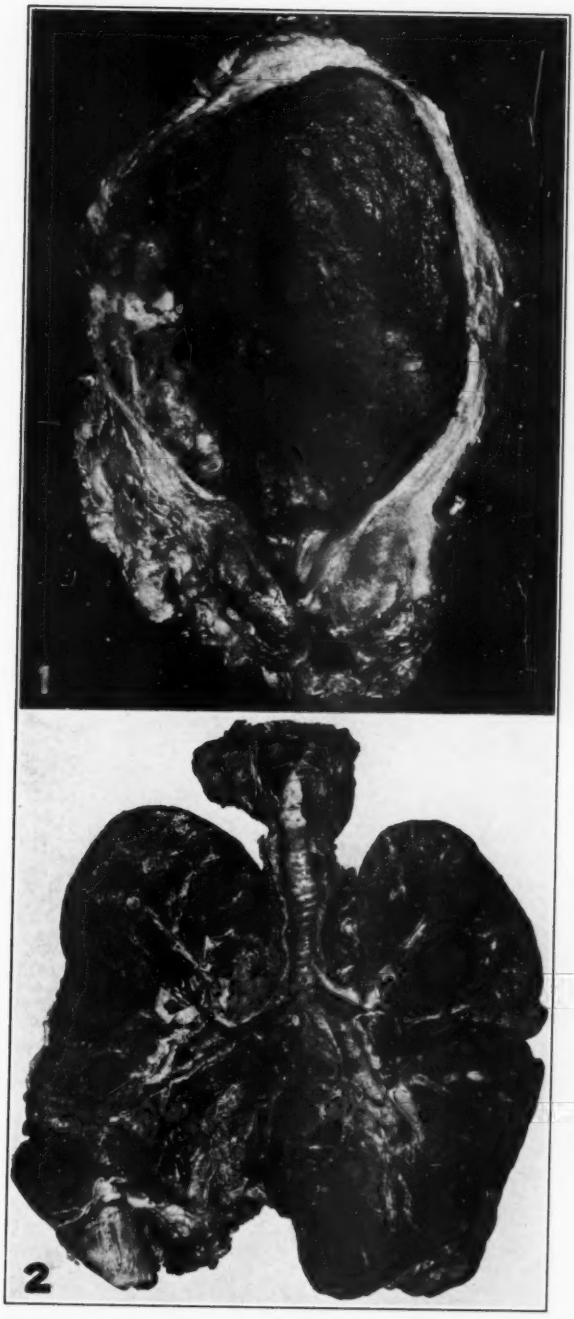
DESCRIPTION OF PLATES

PLATE 113

FIG. 1. Urinary bladder opened anteriorly to show the primary tumor mass involving the entire right lateral wall and a greater portion of the posterior wall. The tumor mass extends from the neck of the bladder far into the fundal region. Areas of hemorrhage are seen in the cross section of the right lateral wall in the fundal region.

FIG. 2. The lungs have been hemisected and the cut surfaces of the anterior halves are shown. Note the numerous, large, well circumscribed projecting tumor nodules scattered throughout the parenchyma.

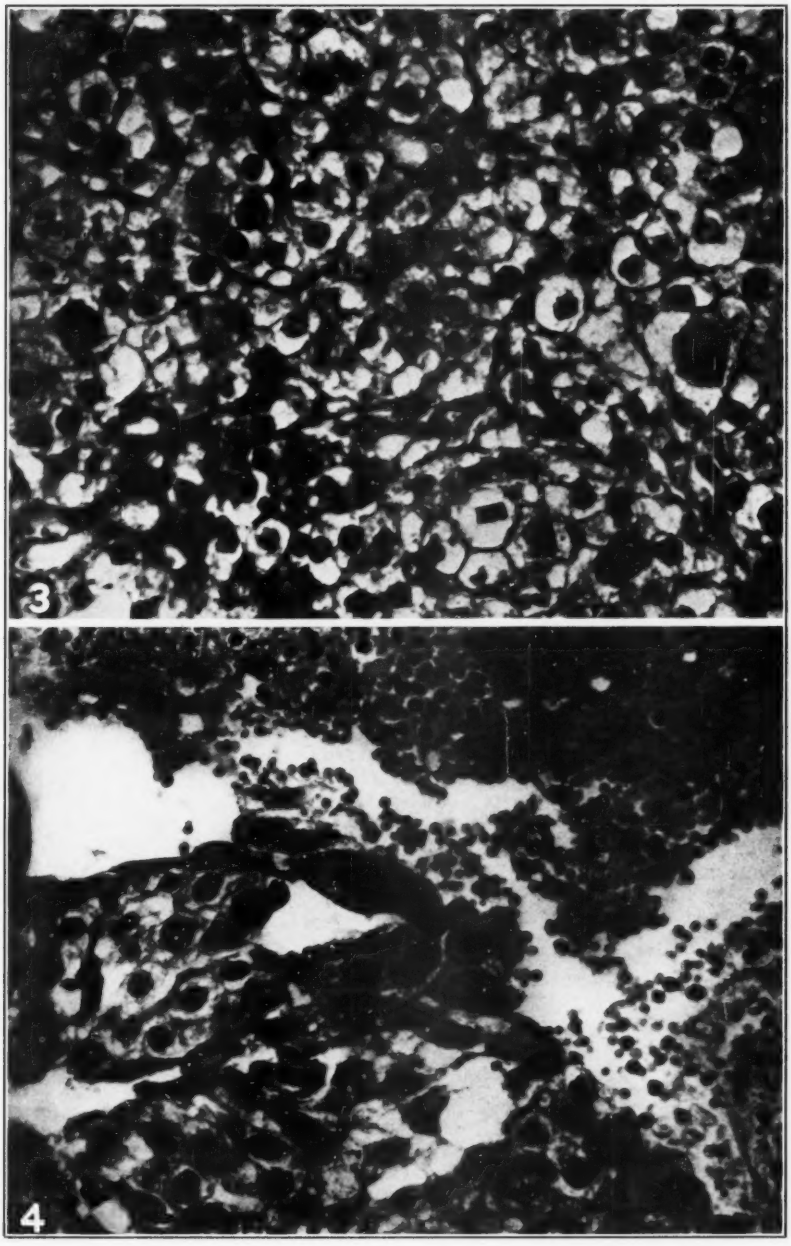


Weinberg Chorionepithelioma of Bladder

PLATE 114

FIG. 3. Microphotograph of the primary tumor of the bladder showing a representative area composed of Langhans' cells. × 620.

FIG. 4. Microphotograph of primary bladder tumor showing nests of syncytial cells and an area of hemorrhage. × 620.



Weinberg Chorionepithelioma of Bladder

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